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The Fate and Effects of Crude Oil Spilled on Subarctic Permafrost Terrain in Interior Alaska

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This study was conducted to determine both the short- and long-term effects of spills of hot Prudhoe Bay crude oil on permafrost terrain in subarctic interior Alaska. Two experimental oil spills of 7570 liters (2000 gallons) each on $500 m^2$ test plots were made at a forest site underlain by permafrost near Fairbanks, Alaska. The oil spills, one in winter and one in summer, were conducted to evaluate their effect during these two seasonal extremes. Oil movement, thermal regime, botanical effects, microbiological responses, permafrost impact, and composition of the oil in the soil were monitored for two years.

The results indicate that oil movement during the winter spill occurred within the surface moss layer beneath the snow. In the summer spill, movement of the oil was primarily below the moss in the organic soil. The oil movement in the summer spill was more rapid, moving 30 m downslope in the first 24 hours and 41 m total through the summer. The oil in the winter spill moved only 18 m downslope in the first day and stopped. Remobilization occurred in the spring allowing the oil in the winter spill to move an additional 17 m. The total area affected by the summer spill was nearly one and one-half times as large as the winter spill ($303 m^2$ vs $188 m^2$ or $40 m^2/m^3$ of oil vs $24 m^2/m^3$ of oil.

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THE FATE AND EFFECT OF CRUDE OIL SPILLED ON SUBARCTIC PERMAFROST TERRAIN IN INTERIOR ALASKA

by

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FOREWORD

Effective regulatory and enforcement actions by the Environmental Protection Agency would be virtually impossible without sound scientific data on pollutants and their impact on environmental stability and human health. Responsibility for building this data base has been assigned to EPA's Office of Research and Development and its 15 major field installations, one of which is the Corvallis Environmental Research Laboratory.

The primary mission of the Corvallis Laboratory is research on the effects of environmental pollutants on terrestrial, freshwater and marine ecosystems; the behavior, effects and control of pollutants in lakes and streams; and the development of predictive models on the movement of pollutants in the biosphere.

This publication reports results of a study to determine the effects of crude oil spills on permafrost terrain in subarctic interior Alaska.

Thomas A. Murphy, Director
Corvallis Environmental Research Laboratory

Abstract

This study was conducted to determine both the short and long-term effects of spills of hot Prudhoe Bay crude oil on permafrost terrain in subarctic interior Alaska. Two experimental oil spills of 7570 liters (2000 gallons) each on 500 m^2 test plots were made at a forest site underlain by permafrost near Fairbanks, Alaska. The oil spills, one in winter and one in summer, were conducted to evaluate their effect during these two seasonal extremes. Oil movement, thermal regime, botanical effects, microbiological responses, permafrost impact, and composition of the oil in the soil were monitored for two years.

The results indicate that oil movement during the winter spill occurred within the surface moss layer beneath the snow. In the summer spill, movement of the oil was primarily below the moss in the organic soil. The oil movement in the summer spill was more rapid, moving 30 m downslope in the first 24 hours and 41 m total through the summer. The oil in the winter spill moved only 18 m downslope in the first day and stopped. Remobilization occurred in the spring allowing the oil in the winter spill to move an additional 17 m. The total area affected by the summer spill was nearly one and one-half times as large as the winter spill (303 m^2 vs 188 m^2 or 40 m^2/m^3 of oil vs 24 $m^2/m^3$of oil).

The heat of the initial spilled oil had little measureable effect on the underlying frozen soil at the time of the winter spill or on the depth of thaw in the summer spill. However, significant changes in depth of thaw occurred following two full thaw seasons. The greatest increases in thaw depths occurred in areas where the surface was blackened by oil.

Chemical studies have shown that evaporation of volatile components is the most significant natural weathering process in the first two years. C_1 and C_2 components were lost from surface oil in the first 24 hours with only small concentrations of components smaller than C_8 present after five months. There was somewhat slower evaporation of volatiles from oil carried deeper in the soil profile. Changes in composition usually attributable to microbial degradation have not been observed.

The indigenous soil microbial populations responded differently to winter and summer oil applications. The responses ranged from inhibition to stimulation, with stimulation of growth and activity appearing to predominate.

Vegetation showed both immediate and long term damage effects from the oil. Damage was greatest near the top of the slope and in surface oil blackened areas. Deciduous species showed damage more rapidly than evergreen species. Additional black spruce died up to two years after the spills.

This report was submitted in fulfillment of grant number EPA-IAF-D7-0794 under the sponsorship of the U.S. Environmental Protection Agency. This report covers a period from 1975 to 1978 and work was completed as of October 1979.

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SUMMARY

In February and July, 1976, two experimental 7570ℓ (2000 gal) oil spills of hot Prudhoe Bay crude were conducted in subarctic interior Alaska in the Caribou-Poker Creeks Research Watershed. The following types of information were obtained during the subsequent three year study:

1. The movement of the oil downslope with time
2. The movement and current presence of oil in the soil profile
3. The change in oil composition with time after spillage
4. The responses of various soil microbiological populations
5. The botanical effects of oil contamination

The following results and conclusions were obtained.

1) There are distinct differences in the effects of a winter oil spill and a summer oil spill.

2) In both winter and summer spills, oil flowed downslope following the microtopography of the site for distances of 35 and 41.5 m, respectively. The total area impacted by the summer spill was nearly one and one half times as large as the winter spill (303 m^2 vs 188 m^2). Downslope movement in the summer spill was primarily in the organic soil. In the winter spill, initial movement occurred beneath the snow, over and through the moss layer, resulting in a much larger area of surface flow (76.3 m^2), relative to the summer spill (30.3 m^2), respectively. Oil movement stopped after one day in the winter spill. The oil then remained immobilized until snow melt when it moved further downslope, this time generally in the organic soil.

3) The total area impacted by these spills was surprisingly small primarily due to the ability of the thick moss layer and organic soil to absorb large quantities of oil. In addition, evaporation of volatiles reduced the volume and mobility of the oil. The average oil content in the impacted areas for the winter and summer spills was 41 and 25 l/m^2, respectively (or 24 and 40 m^2/m^3 of oil).

4) The location of oil in the soil profile differed in the two spills. In the upslope portion of the winter spill plot, the oil resides primarily in the moss and organic soil. Further downslope, where movement occurred after spring breakup, the oil resides primarily in the organic soil. In the upslope portion of the summer spill, most of the oil is in the organic soil, but oil has penetrated as deep as 8 cm into the mineral soil. In areas further downslope in the summer spill, the oil is present in a narrow band in the organic soil.

5) The initial heat input of the hot crude oil did not significantly affect either the frozen soil surface in the winter spill or the depth of thaw in the summer spill.

6) After two full thaw seasons, increased depths of thaw have been observed in the oiled area, with the greatest thaw below visibly oiled (blackened) surface areas. If this trend continues, thermokarsting and reduction in slope stability could result.

7) Compositional change in the spilled crude oil during the first two years is primarily a result of evaporation of volatiles. No observable translocation of water soluble components was found below the area of physical oil movement. The type of oil degradation usually attributed to microbial activity was not observed in the first two years after spillage.

8) The oil will probably remain largely intact for long periods in the soil unless some measures are used to accelerate oil degradation.

9) During the first growing season after the winter spill, the filamentous fungal population was inhibited whereas the heterotrophic bacterial population was stimulated. After the summer spill, a brief initial depression of both the filamentous fungal and bacterial populations occurred but was followed by a general enhancement. In both oil spill plots, denitrifying, proteolytic, oil-utilizing and cellulose-utilizing microorganisms (including yeasts) were favorably affected by the oil spills.

10) During the second and third growing seasons following the oil spills, the filamentous fungal populations were inhibited in both oiled plots. Inhibition was greater in the winter than in the summer plot. The heterotrophic bacteria and yeasts were stimulated in both oiled plots, with greater stimulation in the summer plot. Numbers of denitrifying bacteria, oil-utilizing bacteria and oil-utilizing yeasts in the oiled plots remained elevated.

11) After an initial decrease 24 hours after the oil spills, in vitro soil respiration generally increased in both oiled plots relative to the control for the duration of the study. Soil respiration in situ* increased in the oiled plots only in areas where plants killed by the oil were not removed. This indicates that the increased substrate from dead plant material is at least partially responsible for the increased soil respiration.

12) The effects of crude oil on indigenous soil microbial populations and their activities appear to be both direct and indirect. The filamentous fungal population may be inhibited by toxic crude oil components and reduced aeration while other microbial populations and in vitro**

---

* In place, on site

** In the laboratory

soil respiration may be stimulated by the addition of carbon substrate in the oil and in plants damaged or killed by the oil.

13) Vegetation mortality was highest within areas of surface flows in the upslope portions of the plots. Damage to vegetation was delayed and reduced in areas which were both further downslope and impacted by subsurface flows.

14) In general, deciduous species showed the most rapid injury. Evergreen species displayed more delayed symptoms. Injuries have continued to appear up to the present with additional mortality of black spruce in the winter spill site through the third growing season.

15) Rooting characteristics, as well as above ground growth form, significantly influence sensitivity to the oil. Cottongrass tussocks, with their vertical rooting habit, appear to be the most oil resistant species on this site.

16) There is no evidence of recovery by vegetation in areas of surface flows in these spills. Other crude oil spills in Alaska have shown some vegetative recovery within the same time frame, possibly because of greater soil moisture.

17) Because of the complex interactions of the effects of crude oil spills, a variety of scientific disciplines is necessary to assess environmental impact.

INTRODUCTION

During the last 15 years, oil exploration and development, spurred on by increasing demand for oil and declining petroleum reserves in temperate regions, has been increasing in the Arctic and Subarctic. One large pipeline for transporting crude oil, the Trans-Alaskan Pipeline System (TAPS), was completed in 1977 and other smaller ones have been constructed in Canada and the Soviet Union. These projects have resulted in spills of crude oil and the inevitability of more spills remains.

Regulatory agencies and scientists became interested in ascertaining the environmental effects of petroleum spills as soon as development of petroleum reserves began in the northern regions. In the early seventies a number of small scale studies were begun to document the effects of spills, (Deneke _et al_., 1975; Hutchinson _et al_., 1974) while other studies examined biological recovery after spills (Hunt, 1972; Cook and Westlake, 1974; McGill, 1977). Other individuals recorded the effects of refined petroleum spills along a military pipeline in Alaska (Deneke _et al_., 1975; Rickard and Deneke, 1972; Hunt _et al_., 1973). However, before 1975 only one study, in the Mackenzie Valley of Canada, had attempted to experimentally determine the effects of larger petroleum spills (MacKay _et al_., 1974; Hutchinson _et al_., 1974; Cook and Westlake, 1974). Recently a collection of articles summarizing past and ongoing oil spill research in Alaska, including interim reports of the research presented here, have been published (Arctic 31(3), 1978).

In 1975 the Alaskan Projects Office of the U. S. Army Cold Regions Research and Engineering Laboratory (USACRREL) received funding from the U. S. Environmental Protection Agency, Arctic Environmental Research Station, to study the fate and effects of crude oil spilled on permafrost terrain. This study was specifically designed to simulate the size and location of an actual crude oil spill from a functioning pipeline. An open black spruce stand, representative of interior subarctic Alaska, was selected in the Caribou-Poker Creeks Research Watershed. Two spill plots were designated and 7570 ℓ (2000 gal) of hot (57°C) Prudhoe Bay crude oil were applied to each. The spills, one in winter and one in summer, were conducted in order to assess differences in behavior and impact of the crude oil at the two temperature extremes of the subarctic climate.

There were four overall objectives of the study:

1) to document the physical effects of crude oil spills on black spruce forest, in the interior of Alaska, emphasizing the mode of transport, area of impact with time, and effects on the underlying permafrost;

2) to determine the fate of petroleum, once spilled in subarctic terrestrial environments;

3) to evaluate the effect of crude oil spills on soil microbial populations and their activities;

4) to evaluate the effects of crude oil spills on vegetation.

In order to meet these multidisciplinary objectives the following personnel were engaged on the project:

1. Geologist: to determine the physical extent of oil spread and thermal effects on underlying permafrost (Charles Collins);

2. Engineer: to determine the factors influencing the thermal regime of the soil and design of spill systems (Terry McFadden);

3. Chemist: to determine the changes in the chemical composition of the petroleum over time and over the extent of the spill (Thomas Jenkins);

4. Microbiologist: to determine the response of soil microbial populations to winter and summer oil spills (Elena Sparrow and Charlotte Davenport);

5. Botanist: to determine the effects of the crude oil upon the existing vegetation species and the extent of their recovery (Larry Johnson).

METHODS

Site Description

The study site lies in the lower reaches of the Caribou-Poker Creeks Research Watershed, 48 km north-east of Fairbanks, Alaska (Fig. 1). The site is about 300 m above sea level and situated on a moderate (7-8%) west-facing slope. Two study plots, each 10 m by 50 m with the long axis downslope, were established for oil application. Control areas were designated nearby. The closest stream is Poker Creek, 800 m downslope of the site. An abandoned water diversion ditch lies between the study site and Poker Creek, guarding against inadvertent oil contamination.

The study site is an open black spruce (*Picea mariana* (Mill.) Britt, Stearns & Pogg.) stand (Fig. 2) with a shrub understory of Labrador tea (*Ledum decumbens* (Ait.) Hult.) and *Ledum groenlandicum* ((Oeder) Hult.), resin birch (*Betula glandulosa* Michx.), and blueberry (*Vaccinium uligonosum* L.). A few scattered willows (*Salix* spp.) occur in the area. Mosses and lichens cover 50% or more of the ground surface and various herbs can be found. Cottongrass tussocks (*Eriophorum vaginatum* L.) have scattered distribution but are of local importance (Troth *et al*., 1975).

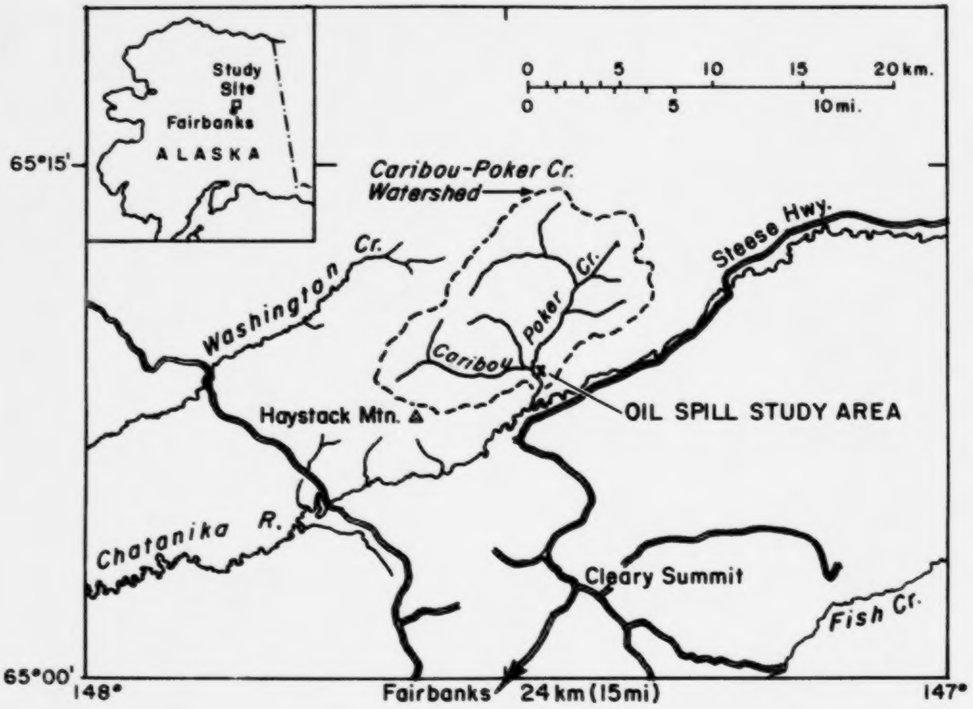

Study Site
Fairbanks
ALASKA
0 5 10 15 20 km.
0 5 10 mi.
65°15'
Caribou-Poker Cr. Watershed
Steese Hwy.
Washington Cr.
Cr.
Poker
Caribou
OIL SPILL STUDY AREA
Haystack Mtn.
Chatanika R.
Cleary Summit
Fish Cr.
65°00'
148°
Fairbanks 24 km (15 mi)
147°

Figure 1. Location of oil spill site near Fairbanks, Alaska.

Elevated crosswalks were installed at 5 m intervals across the treatment plots to allow direct access to the entire surface of the plots with minimum disturbance from surface trampling (Fig. 2).

The soil on the site is typical of the Saulich series found in the lower slopes of the watershed (Rieger et al., 1972). A typical profile consists of 25 to 5 cm of moss (O_1), 8 to 0 cm of reddish-brown peat (O_2), 0 to 8 cm of brown mixed organic and silt loam (A_1) and greyish silt loam extending to permafrost (C_2) (Fig. 3). The soil, a histic pergelic cryaquept, is poorly drained with an active layer depth of 15 cm to 50 cm depending on the thickness of the organic mat and proximity to shrubs and trees. The pH of both the organic and mineral soils is acidic, generally about pH 4 (Troth et al., 1975; Rieger et al., 1972).

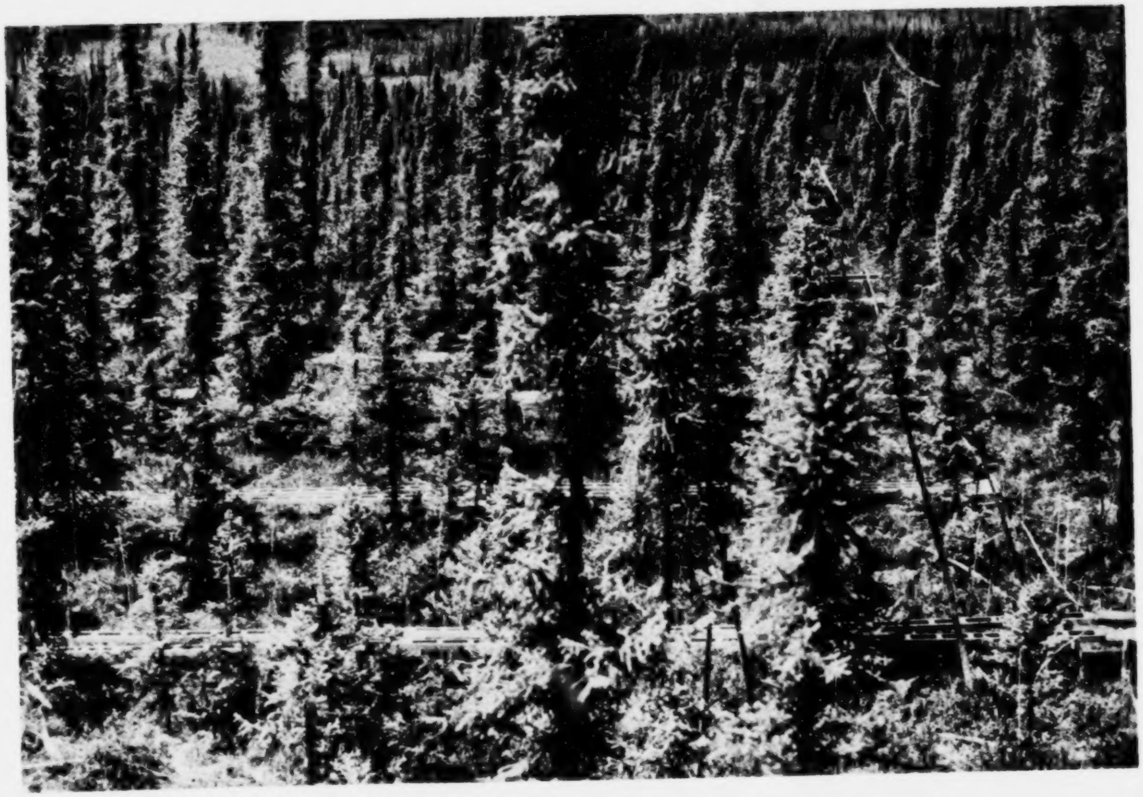
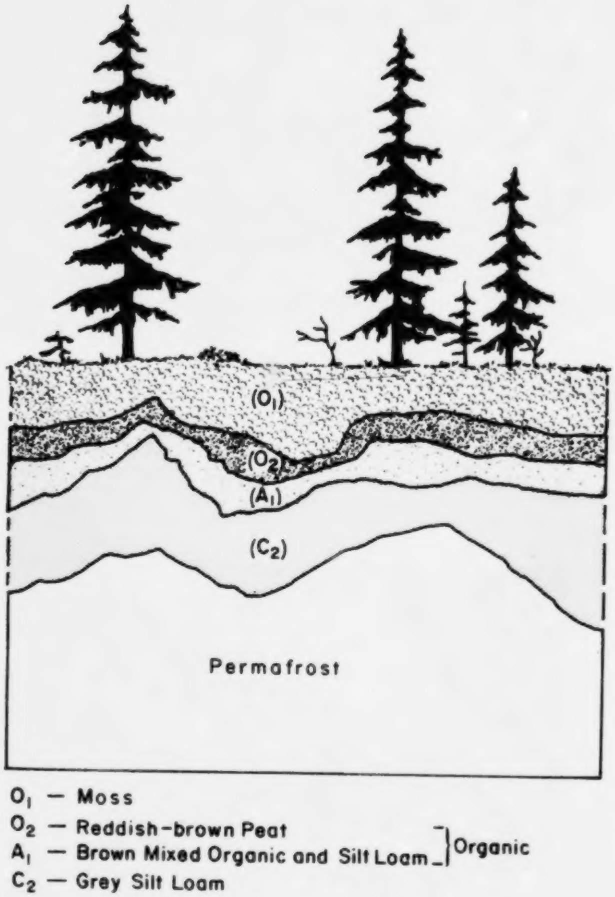


Figure 2. View looking downslope prior to oil application on summer spill plot (note elevated crosswalks installed for access to plot).

Oil Application

The crude oil for the study was obtained from Prudhoe Bay and hauled by truck in 55 gal drums to an area near the site. The oil was transferred to a 2000 gal tank and transported to the spill site on a large tracked vehicle. The oil was heated using heat tapes, to 57°C, in a closed system to preserve the volatile components, and was spilled through a 5 m length of perforated pipe (Fig. 4) at about 170 l/min over a 45 minute period. The winter spill was conducted on 26 February 1976 at an ambient air temperature of about -5°C (23°F). The summer spill



(O$_1$)
(O$_2$)
(A$_1$)
(C$_2$)
Permafrost
O$_1$ — Moss
O$_2$ — Reddish-brown Peat
A$_1$ — Brown Mixed Organic and Silt Loam
Organic
C$_2$ — Grey Silt Loam

Figure 3. Typical profile of Saulich soil at Caribou-Poker Creek spill sites.

was carried out on 14 July 1976 to approximately coincide with the peak of the growing season and maximum yearly temperatures. The air temperature during the summer spill was 25°C (77°F). In each test 7570 ℓ (2000 gal) of oil were spilled.

Physical Characterization

The rate of oil flow downslope following the spill was determined by probing with wooden dowels at predetermined locations on a grid with one meter intervals (Fig. 5). The presence of the crude oil was readily

Figure 4. Oil being spilled along a 5 m length of perforated pipe at winter spill plot (note in the background the tank in which the oil was heated prior to the spill).

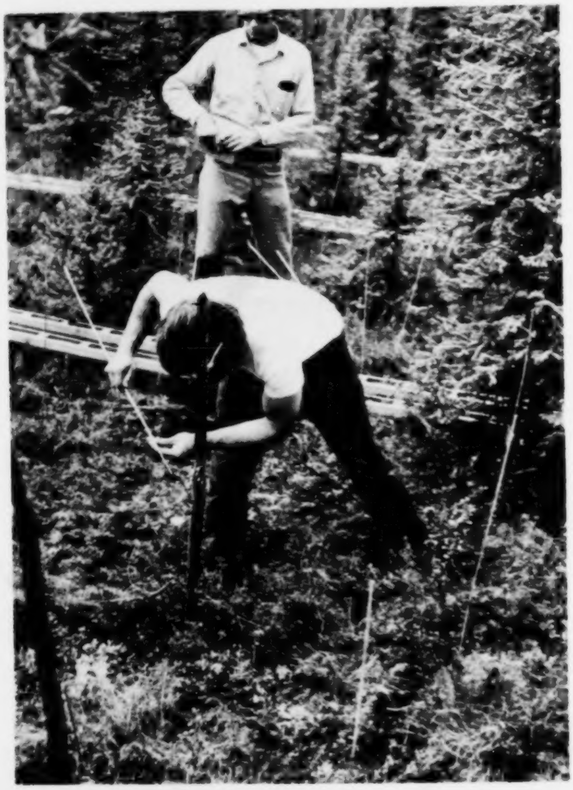


Figure 5. Following oil flow downslope on the summer plot by visually inspecting wooden dowels.

discerned by sight and smell, and confirmed by UV-fluorescence (Deneke *et al.*, 1975) when questionable.

In July 1978, small pits were excavated at selected sites on the two spill plots to determine the vertical distribution of the oil in the soil profile. Penetration of the oil was determined visually.

Thermal Characterization

Vertical thermocouple arrays were installed with individual thermocouples at five levels (See Appendix, Figures A1 and A2, Table A1).

To determine changes in the depth of thaw in the plots, probings were conducted during the thaw periods. Six cross sections were laid out at 1, 3, 6, 9, 14 and 20 m downslope from the spill point in each plot and frequent probings were taken at one meter intervals each summer.

Oil and Oily Soil Characterization

Initial oil samples were obtained at the time of the spills, cooled rapidly to preserve their integrity with respect to volatile components, and characterized along with samples collected later by the following techniques:

1) headspace analysis for volatiles by gas chromatography;
2) silica gel/alumina column chromatography to obtain major oil components (alkanes, aromatics, asphaltenes, and NSO's);
3) characterization of the alkane fractions by capillary flame ionization gas chromatography (Apiezon L and Dexsil SCOT columns);
4) Characterization of all oil fractions by scanning infrared spectroscopy.

Soil cores were collected initially, and then periodically over a three-year period. Each sample was taken with a 5.7 cm diameter corer, divided visually into overlying moss (0_1), brown organic layer (0_2 and A_1) and greyish-brown silt loam (C_2), and individually stored in prewashed glass canning jars. The samples were iced immediately and kept frozen until analysis. Additional samples were collected in July, 1978 from small soil pits excavated at selected points in the contaminated areas.

The soil samples were analyzed as follows. A subsample was taken, placed in a scintillation vial, sealed with silicone rubber septum material, and equilibrated at room temperature (approximately 22°C) for 24 hours. The headspace was sampled with a gas-tight syringe, and analyzed by gas chromatography (McAuliffe, 1971). Gas chromatographic analysis was conducted as follows:

- analytical column - 5% Durapak, Carborax 400 on Porasil C (9 ft x 1/8 in.)
- oven temperature - programmed from -20°C to 150°C at 10°/min.
- flow rate - 20 ml/min of helium carrier gas
- detector - flame ionization

The remainder of the soil sample was manually extracted with chloroform (Mallinckrodt, nanograde). For oily soils, extractions were continued until the color of extract was visually similar to that from a control soil (up to 15 individual extractions). The extracts were combined and the chloroform removed by evaporation. The residue, containing material C_{15} and greater, was determined gravimetrically.

The dry weight of the soil was obtained by oven drying the extracted soil at 105°C for at least 24 hrs. Percent oil was calculated by dividing the chloroform extractable residue by the dry weight of soil.

The extracted oil was fractionated as follows. The asphaltene material was removed by precipitation with pentane and determined gravimetrically. The remainder of the material was fractionated by silica gel-alumina column chromatography in a manner similar to that described by Bailey et al., (1973). In our study, the column was sequentially eluted with 100 ml of pentane, 150 ml of benzene, and 100 ml of methanol (Mallinckrodt, nanograde) with the amount of material in each fraction determined gravimetrically.

The alkane fraction was further characterized by gas chromatography. Several 50 ft x 0.02 in. SCOT columns were used including Dexsil 300 GC and Apiezon L. Identification of individual peaks was initially obtained by comparison to standard samples and confirmed later by gas chromatography/mass spectroscopy (HP 5992). Ratios of pristane (2, 6, 10, 14 tetramethylpentadecane) to n-C_{17} (heptadecane) were obtained by measuring the peak heights of these components in the Apiezon L chromatograms. The peak heights (and thus their ratio) are not absolute measurements and are dependent on the degree of separation achieved for these two components. The higher numbers reported elsewhere for this ratio (Jenkins et al., 1978) were obtained on a different analytical column of the same variety.

Infrared spectra of all the fractions, obtained by silica gel-alumina fractionation, were determined on a PE 167 scanning IR spectrophotometer.

Inorganic analysis of soil was done on dried, sieved (2mm), mineral (C_2) and organic (0_2, A_1) soils which had been treated with chloroform to remove the oil. Soil pH was obtained with a digital pH meter (glass electrode) on a 5:1, distilled water to dry soil, suspension according to Jackson (1958). Exchangeable Ca^{++}, Mg^{++}, Na^{+} and K^{+} were obtained by further extraction of a subsample of the dried soil with 1N ammonium acetate (Jackson, 1958) and determination by atomic absorption spectrometry (PE 303). Exchangeable ammonium was determined on a second soil subsample by extraction with 1N KCl and determination by the automated phenate method (Technicon Industrial Method #98-70W, 1973). Soil nitrate was determined in the KCl extract by the automated cadmium reduction method (Technicon Industrial Method #271-73W, 1973). All values for Ca^{++}, Mg^{++}, Na^{+}, K^{+}, NH_4^{+} and NO_3^{-} are reported on a dry weight soil basis.

Soil water samples were collected in July, 1977. These samples were obtained by digging small pits on each spill plot (and in a control area), just beyond the farthest downslope point where visible oil was detectable in the soil profile, and allowing water to collect in the pits. Several of these water samples were analyzed for total organic carbon by combustion infrared analysis using a Beckman 915 Total Organic Carbon Analyzer. The dissolved volatile material ($<C_8$) was determined by a modified stripping technique according to Leggett (1979).

Soil Microbiological Methodology

a. Collection and processing of soil microbiological samples:

Soil samples were collected from the A_1 horizon (zone of organic matter accumulation) and from the upper 5-7 cm of the C_2 horizon in each test plot. Soil sampling in the oiled plots was confined to the first 5 to 10 m downslope from the line of oil application, where the concentration of oil appeared to be highest. The soil sampling dates in 1976 were 27 February, 17 June, 14 July, 12 August, and 27 September; in 1977, sampling dates were 9 June, 5 July and 1 August; in 1978 the dates were 27 June and 12 July. Each oiled plot was first sampled within 24 hours after oil was first applied. During the winter sampling (February), a gasoline-powered corer was used to obtain soil cores (frozen). During the growing season (June to September), soil samples were taken with a hand-operated corer. Several soil cores were randomly taken from each plot. Soil cores were visually examined, then separated into A_1 and C_2 sections and placed in separate containers. The soil cores were kept at 4°C and processed within 24 hours after sampling. During the sample processing, plant roots were removed, the soil cores were composited, and subsamples were then taken for microbial and chemical analyses. All dilutions and plating for microbial analyses were done in a 10°C environmental room to minimize possible heat injury to psychrophilic microorganisms.

b. Enumeration of soil microorganisms:

Changes in microbial numbers were monitored using dilution plate count (surface-spread) and Most-Probable-Number (MPN) techniques. Plate count agar (Difco) with cycloheximide (50 mg/l) plus additional agar (5 g/l) was used for counting heterotrophic and anaerobic bacteria. Martin's medium (Martin, 1950) was used for enumerating filamentous fungi and yeasts. A gelatin medium and the procedure described by Rodina (1972) was used to count proteolytic bacteria. A nitrate medium and a five-tube MPN procedure (Alexander, 1965) was employed to determine the abundance of denitrifying bacteria. Five replicate plates or five tubes per dilution were used for microbial enumeration except for the anaerobes which were done in triplicate. Incubation at 20°C for one week and 4°C for two weeks was used for all groups except the denitrifiers which were incubated for one month at both temperatures. Anaerobiosis for the determination of the abundance of anaerobic bacteria was attained by evacuating Brewer jars, flushing with nitrogen gas containing 5% carbon dioxide, and using copper sulfate treated steel wool (Parker, 1955).

The enumeration of oil-utilizing microorganisms was a two-step process using a five-plate MPN procedure. Initially, samples were plated on a modified silica gel medium containing 1% Prudhoe Bay crude oil. After one month incubation (at both 4°C and 20°C), these plates were replicated onto plate count agar to estimate the bacterial population and onto Martin's medium to estimate the fungal populations. The replica plates were subsequently incubated, one week for the 20°C plates and two weeks for the 4°C plates. The presence of one or more colonies on the replica plate constituted a positive test.

Silica gel plates were prepared according to the procedure of Funk and Krulwich (1964) with the mineral salts medium of Bushnell and Haas (1941). Our modification was the addition of a sufficient quantity of colloidal silica (Cab-O-Sil MS obtained from Cabot Corp., Boston, Mass.) to the mineral medium to make a final concentration of 1% colloidal silica in the gel plates. The colloidal silica was added to help maintain dispersion of oil in the gel plates. In making the silica gel plates, the components were autoclaved separately and allowed to come to room temperature before use. Sterilized oil (Robertson _et al_., 1973) was added to the mineral-colloidal silica medium and the medium was mechanically mixed to disperse the oil. Then the other components were added, the mixture shaken, and the plates poured. Gelling occurred within one minute, which was important in maintaining dispersion of the oil in the plates.

The estimation of the numbers of cellulose-utilizing microorganisms was also done on silica gel plates using the five plate MPN procedure. The silica gel plates for this determination contained 1% cellulose (Sigmacell-Type 20^{R}) as a sole carbon source and the colloidal silica was not added.

c. Soil respiration measurements:

_In vitro_* respiration rates of soil samples were determined by measuring carbon dioxide evolution rates in biometer flasks (Bellco

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* In the laboratory

Glass, Inc.) according to the procedure of Johnson and Curl (1972). Five replicate determinations were done on samples from each soil horizon in each test plot with incubation at 4°C and 20°C.

In situ* soil respiration was determined by measuring carbon dioxide evolution rates in the field. Open-ended cylinders (7.5 cm diameter) were sunk into the test plots. In the control plot, the live moss plant layer was clipped before the ten cylinders were embedded, to ensure that only litter, root, and soil respiration were being measured. In the oiled plots, two sets of cylinders were embedded, one with the killed moss layer removed (5 cylinders per plot) and one with the killed moss layer intact (10 cylinders per plot). Carbon dioxide evolution rates were determined according to Coleman (1973), with the exception that the alkali jars were suspended (from the rubber stoppers used to cap the cylinders) instead of being placed on the soil surface. Control cylinders containing only jars of alkali were run simultaneously during the 24 hour-period that respiration rates were being measured.

d. Soil water, pH, and oil determination in soil microbiological samples:

Water content of soil samples was determined using the gravimetric method described by Gardner (1965). The pH of fresh soil samples was measured using a glass electrode in a 1:1 soil-distilled water suspension (Peech, 1965).

Oil content in soil samples was determined gravimetrically on benzene extracts of soil. Three replicates of approximately 15 g of wet soil from each soil sample were dried at 105°C for 24 - 48 hr. Each replicate soil sample was then extracted 4 to 5 times with 50 ml portions of benzene. The extracts were combined, evaporated to dryness in a fume hood at room temperature, and the residue weighed. The percent dry residue obtained from the unoiled soil was subtracted from the percent dry residue from oiled soil to give corrected percent oil from oiled plots. The weight of the oil contributing to the dry weight of soil was taken into consideration in the calculation of the oven dry weight of oiled soils.

e. Statistical analyses of microbiological data:

Counts of microorganisms and in vitro soil respiration rates are expressed on the basis of oven dry weight of soil. Plate counts, soil respiration rates, and water content (untransformed data) were statistically analyzed using analysis of variance and Duncan's multiple range test (Duncan, 1955). Confidence limits were calculated for the MPN counts. For the comparison of two MPN estimates, non-overlap of the 95% confidence limits was considered indicative of a significant difference between the estimates (Cochran, 1950).

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* In place, on site

Vegetation

Prior to the spill, the vegetation was characterized using a number of one meter square quadrats within both spill plots (Fig. 6). All quadrats were sampled again in July 1978. Data were collected according to methods of Ohmann and Ream (1971) as previously used in the research watershed (Troth et al., 1975). Nomenclature follows Hultén (1968) for herbaceous species, Viereck and Little (1972) for shrub and tree species, Crum et al. (1973) for moss species, and Hale and Culberson (1966) for lichen species.

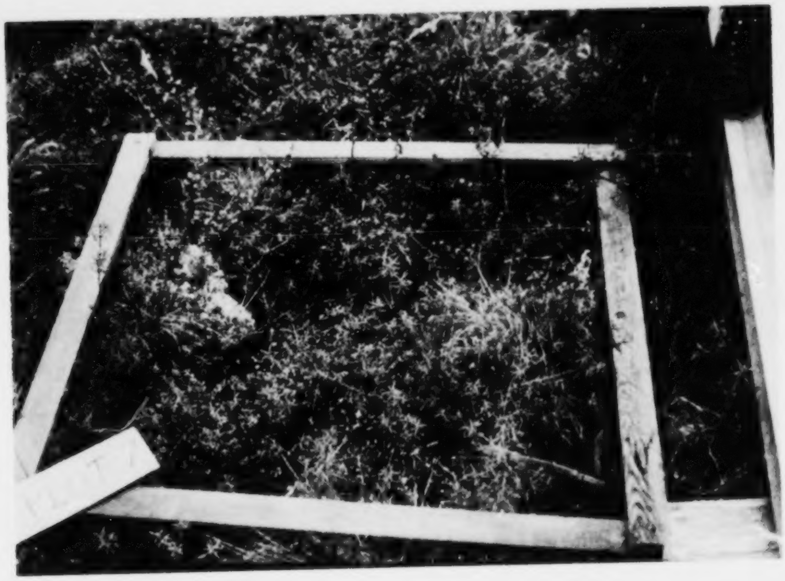

Figure 6. One meter square quadrat used for sampling vegetation.

Pre- and post-spill measurements were made of percentage of cover and frequency of occurrence of vegetation species. The number of stems or individuals was also recorded for some shrub and herbaceous species.

The site was visited during the growing seasons, biweekly during 1976 and 1977 and monthly during 1978, in order to record the time and extent of oil related injuries. Visual estimates of damage were made and photographs were taken of affected quadrats.

Samples of the current year's spruce needles from trees within and outside the winter and summer spill areas were taken in 1975 and 1976. These were analyzed for total nitrogen, phosphorus, and potassium using a Technicon Autoanalyzer to determine if the spills significantly affected nutrient content.

Dr. Arthur Linkins, Virginia Polytechnic Institute, took several soil cores from both the winter and summer oil spill plots during the 1977 growing season. These were used to evaluate ectotrophic root respiration for species including black spruce, blueberry, and Labrador tea. Root respiration rates and respiratory quotients (ratio of carbon dioxide released to oxygen absorbed) were determined in a Gilson differential respirometer using the direct KOH method.

Dead black spruce on the plots were marked in August, 1977. In August, 1978 both plots were re-examined in order to determine if any additional spruce had died. The number of dead spruce was recorded through 1977 and for 1978.

Soil pits were dug within the plots during July 1978 to determine vertical and horizontal root distribution. Root distribution was then compared with oil distribution.

RESULTS AND DISCUSSION

Oil Movement

The winter spill was conducted on 26 February, 1976. The 57°C oil was applied from a 5-m-wide header with 6-mm holes spaced every 10 cm. The oil was spilled on top of a snow pack which was approximately 45 cm in depth. The hot oil rapidly melted holes in the snow, with the snow melting and collapsing one to two meters downslope of the header (Fig. 7).

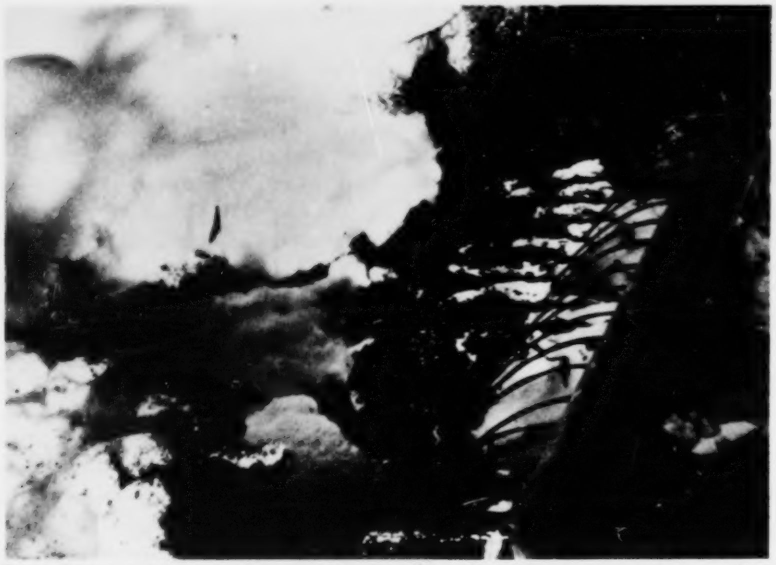

Figure 7. Snow melting near the header during hot crude oil application on the winter plot.

The oil then moved downslope under the snow without disturbing the snow surface. Most of the movement occurred just above and within the moss (O_1) above the frozen organic (O_2, A_1) and mineral (C_2) horizons. Although the oil was not visible from the surface, its presence beneath the snow was obtained by probing and is plotted with respect to time in Figure 8a.

Movement of oil continued at a gradually decreasing rate for 24 hours following the spill before it became immobilized over an area extending 18 m downslope of the header. The oil remained stationary throughout the remainder of the winter and did not remobilize until snow melt in May, 1976. With the onset of warm weather, portions of the oil gradually moved an additional 17 m downslope. The remobilized oil moved beneath the moss layer and was visually undetectable at the surface, in contrast to the initial winter oil movement which occurred over and within the moss layer. The total area affected by the oil is about 188 m^2 or about 24 m^2/m^3 of oil, with an average oil concentration in the impacted area of 41 ℓ/m^2.

The summer spill was conducted on 14 July, 1976 in the same manner as that in the winter spill. A similar spill rate of 170 l/min was used and the oil temperature at the header was also 57°C. As the oil spilled onto the surface, it rapidly penetrated to the peat (O_2) horizon and moved downslope beneath the moss. Oil disappeared from view less than a meter downslope from the spill point and was only visible downslope in surface depressions where pools formed. Oil movement continued rapidly for approximately 24 hours at which time it had moved 28 m downslope. After 48 hours the oil had moved only an additional 6 meters. Oil continued to move downslope until winter freeze up in October, 1976, at which time it had moved another 7 meters. Oil movement plotted with respect to time is shown in Figure 8b.

The total area affected by the summer spill was 303 m^2 or 40 m^2/m^3 of oil, more than one and a half times the area affected by the winter spill. The average concentration of oil in the impacted area was about 25 ℓ/m^2, considerably less than in the winter spill. The areas affected by the oil in both cases compare favorably with the range of 20 to 100 m^2/m^3 of oil predicted by MacKay et al., (1974). However the subsurface flow found in the summer spill differs from that observed by MacKay et al., (1974) where surface flow over water saturated moss occurred. The differences observed are likely a result of a lower moisture content in the moss and upper soil layers of the summer spill which allowed the oil to quickly penetrate to the organic soil where downslope flow occurred. If the moss layer had been very wet at the time of the summer spill, it would probably have caused the oil to float on top of the moss and flow downslope on the surface.

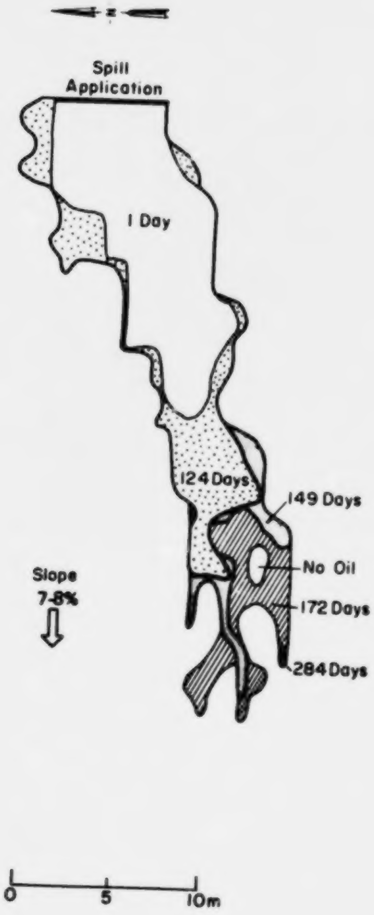
Spill Application
1 Day
124 Days
149 Days
No Oil
172 Days
284 Days
Slope 7-8%
0
5
10m

Figure 8a. Downslope movement of oil following the winter spill.

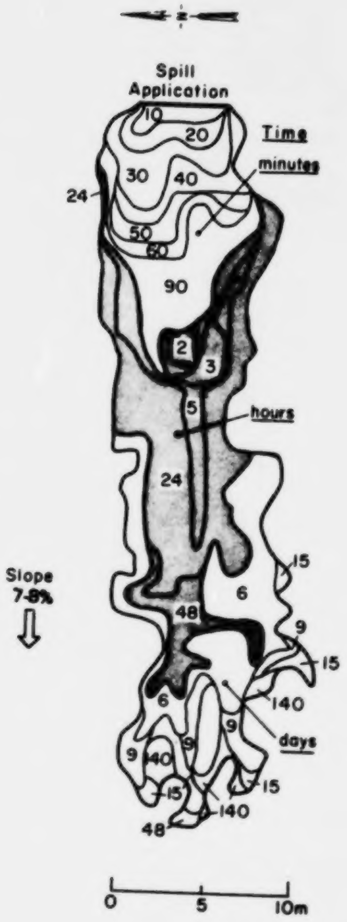
Spill Application
10
20
Time
minutes
30
40
24
50
60
90
2
3
5
hours
24
15
6
48
9
15
140
days
6
9
9
140
9
15
15
140
48
Slope 7-8%
0
5
10m

Figure 8b. Downslope movement of oil following the summer spill.

Figures 9a and 9b are plane views of the summer and winter spills showing the total areas affected by oil and the areas where oil is visibly present on the surface.

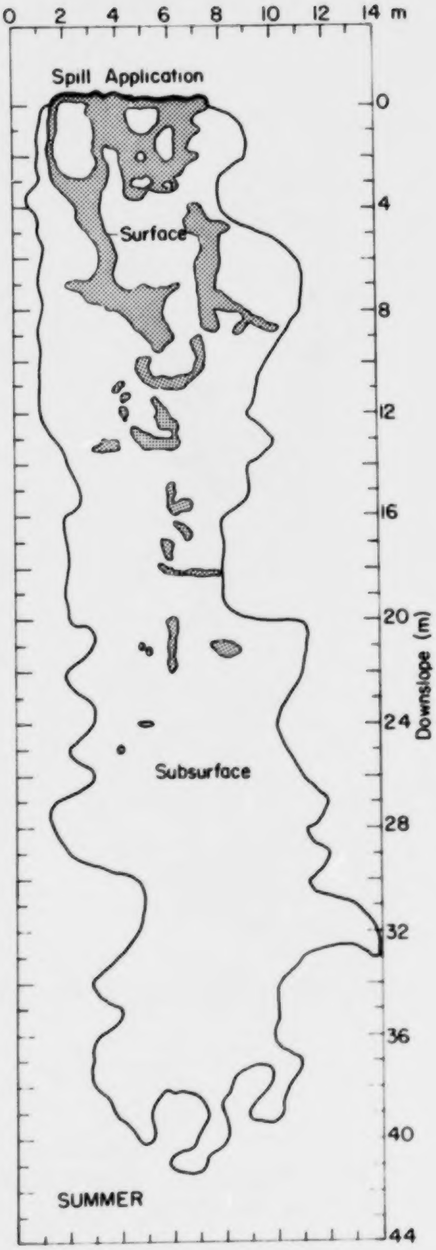
0 2 4 6 8 10 12 14 m
Spill Application
Surface
Subsurface
SUMMER
0
4
8
12
16
20
24
28
32
36
40
44
Downslope (m)

Figure 9a. Summer spill showing surface and subsurface oiled areas.

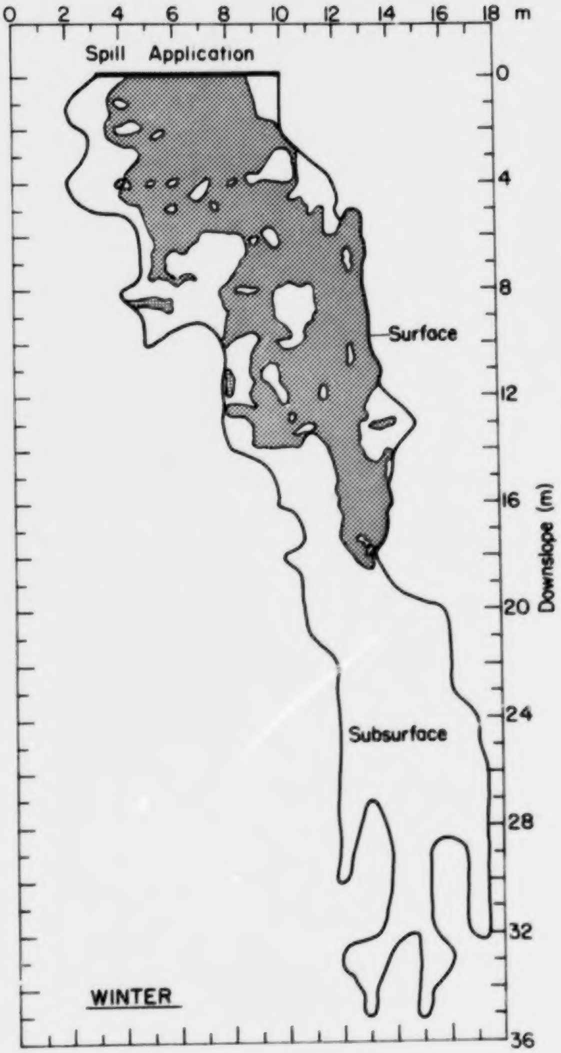
0 2 4 6 8 10 12 14 16 18 m
Spill Application
Surface
Subsurface
WINTER
0
4
8
12
16
20
24
28
32
36
Downslope (m)

Figure 9b. Winter spill showing surface and subsurface oiled areas.

Only 10% of the total summer spill area of 303 m^2 has oil visible on the surface. On the other hand, 40% of the 188 m^2 winter spill has visible surface oil. The difference in visible oil on the surface reflects the different modes of oil movement in the two spills.

In July 1978 the vertical penetration of oil into the soil profile was determined at several points in the two spill plots. A diagram of the location of oil in the soil profile at each site is shown in Figure 10. Five locations were chosen and labeled with site coordinates across slope and downslope (see Figures 9a and 9b for locations) as follows:

- Summer 1-2*, 8-9** (Figure 10a)

  A site with no visible oil on the surface, near the edge of a visibly impacted oil area.

- Summer 4-5, 8-9 (Figure 10b)

  A location in the upper portion of the summer plot which had 80% of the surface visibly impacted by oil.

- Summer 7, 34 (Figure 10c)

  A location near the bottom of the oiled area which was impacted by oil movement several days after the spill.

- Winter 8-9, 11-12 (Figure 10d)

  A site impacted from above at the time of the winter spill which had a heavily oil impacted surface.

- Winter 15, 34 (Figure 10e)

  This location was affected by previously immobilized oil which began moving through the organic layer following spring break-up, several months after the spill.

The diagrams from locations downslope on the summer spill (Fig. 10 a-c) confirm that movement occurred predominantly in the organic layer. Upslope, in the heavily oil impacted zone, oil flowed through the overlying moss and penetrated the mineral soil to a depth as great as 8 cm (Fig. 10b). Oil generally penetrated only 1 cm into the mineral soil further downslope (Fig. 10c). Oil movement upwards from the organic soil into the overlying moss was also reduced downslope to approximately 1 cm.

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* Across slope coordinate (Fig. 9a)

** Downslope coordinate (Fig. 9a)

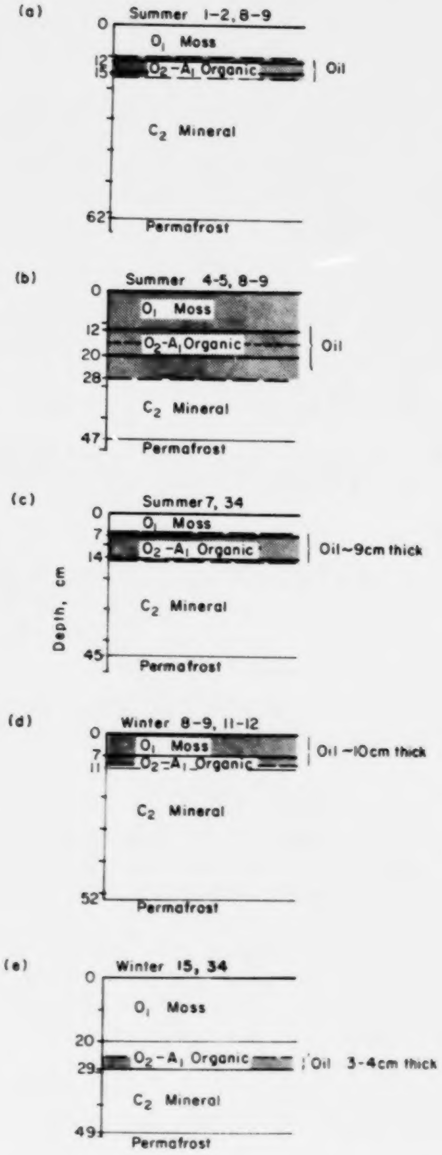

(a)
Summer 1-2, 8-9
0
12
15
62
O1 Moss
O2-A1 Organic
Oil
C2 Mineral
Permafrost
(b)
Summer 4-5, 8-9
0
12
20
28
47
O1 Moss
O2-A1 Organic
Oil
C2 Mineral
Permafrost
(c)
Summer 7, 34
0
7
14
45
Depth, cm
O1 Moss
O2-A1 Organic
Oil~9cm thick
C2 Mineral
Permafrost
(d)
Winter 8-9, 11-12
0
7
11
52
O1 Moss
O2-A1 Organic
Oil ~10cm thick
C2 Mineral
Permafrost
(e)
Winter 15, 34
0
20
29
49
O1 Moss
O2-A1 Organic
Oil 3-4cm thick
C2 Mineral
Permafrost

Figure 10a,b,c,d,e. Vertical penetration of oil into soil profile.

Oil contamination in the first 18 cm of the winter plot occurred from above as shown in Figure 10d. The major portion of the oil in this section was found in the moss layer with significant amounts in the upper part of the organic layer. Little oil was found in the mineral soil in this region.

The distribution of oil in the lower portion of the winter plot was quite different. This area was impacted by remobilized oil after break up. Oil in this region was in a narrow band about 3 cm in thickness within the organic layer (Fig. 10e). No oil was found in either the moss or mineral soil in this section of the plot.

While the average concentrations of oil in the summer and winter plots were 41 and 24 l/m^2 it is clear that this oil was not uniformly spread over the contaminated zone. Areas such as summer 4-5, 8-9 have much higher amounts of oil while those such as winter 15, 34 have very little.

It is useful to compare the physical behavior of these controlled spills with two actual spills from the Trans Alaskan Pipeline System (TAPS) at Valve 7 (19 July, 1977) and Steele Creek (15 February, 1978). At Valve 7 more than 300,000 ℓ (80,000 gal) of crude oil were sprayed under high pressure as far as 1200 m downwind, but the oil only saturated the vegetation within an area 230 m from the valve (Walker et al., 1978). In contrast, at Steele Creek more than 1,900,000 ℓ (500,000 gal) of crude oil sprayed down into the gravel workpad. Although some minor amounts sprayed into the air, most of the oil seems to have flowed on or under the surface. While some oil flowed in the upper organic layers of the soil underneath the snow (similar to the CRREL winter spill) large amounts also flowed over the top of the snow surface. Evidently the oil, which had reached a temperature of only 10°C(50°F) in the pipe, cooled sufficiently before reaching the snow so that it could flow over it. The behavior of these actual spills seems to combine aspects of both the experimental spray spills conducted by Deneke _et_ _al_. (1975) and the experimental point spill studies reported here.

Effects on Permafrost

Very little modification to the underlying frozen ground occurred during the actual spills. During the winter spill, most of the heat of the oil was dissipated by the melting of snow. There may have been some minor thawing of the permafrost table in the upper levels of the summer spill plot at the time of the spill. However, the actual thermal mass of oil, even at 50°C, was negligible compared to the thermal mass of the underlying permafrost. In addition, the oil moved downslope so rapidly that there was little concentration or pooling of oil which could supply enough heat to thaw the permafrost table. Insulation, such as the soil and peat layers above the permafrost, increases the time and the amount of heat required to penetrate to the permafrost.

Oil blackened surface areas have a lower albedo than non-affected vegetation causing the surface to warm above that of non-affected areas. This can result in long term increases in depth of thaw. Figure 11 presents the maximum thaw depths in 1977 and 1978 for cross sections of the two spill plots and a control area at three meters downslope. The greatest thaw depths in the winter and summer plot generally correspond to the surface-oil impacted areas.

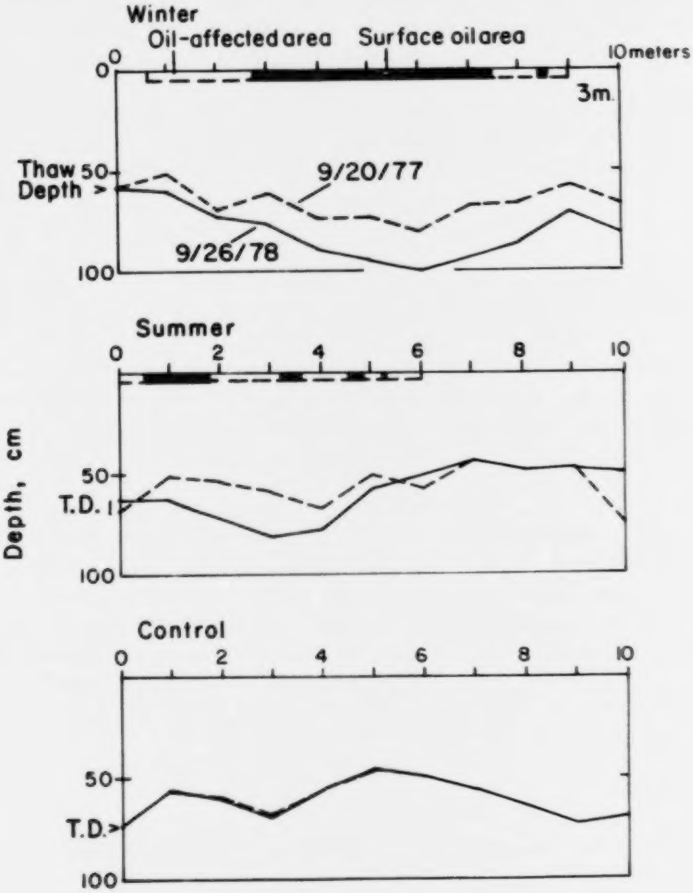


Figure 11. Cross section thaw depths in 1977 and 1978 at 3 meters downslope of oil application.

Table 1 summarizes the average thaw depths of the six cross sections in each of the three plots at 1, 3, 6, 9, 14, and 20 meters as well as the percent change in thaw depths from 1976 to 1977 and from 1977 to 1978. The thaw depth profiles were obtained at thermally equivalent times during each year at near maximum thaw depths. On 28 September

TABLE 1 AVERAGE THAW DEPTH (CM)

		1976	1977	1978	% Change 76-77	% Change 77-78	Average % Change 76-77	Average % Change 77-78
WINTER CROSS SECTIONS	1M	55.4	73.3	87.8	+32.3 %	+19.8 %	21.2 %	13.2 %
	3	53.9	67.4	81.4	+25.0 %	+20.8 %		
	6	54.2	67.4	74.9	+24.4 %	+11.1 %		
	9	49.5	56.1	62.9	+13.3 %	+12.1 %		
	14	44.9	53.4	57.6	+18.9 %	+ 7.9 %		
	20	47.2	52.6	54.7	+11.4 %	+ 4.0 %		
AVERAGE THAW FOR YEAR		50.9	61.7	69.9				
SUMMER CROSS SECTION	1M	48.6	58.0	65.8	+19.3 %	+13.4 %	17.5 %	9.4 %
	3	43.6	57.6	60.3	+32.1 %	+ 4.7 %		
	6	45.2	52.4	60.3	+15.9 %	+15.1 %		
	9	46.6	54.9	62.3	+17.8 %	+13.5 %		
	14	48.1	54.4	57.1	+13.1 %	+ 5.0 %		
	20	51.3	55.4	58.6	+ 8.0 %	+ 5.8 %		
AVERAGE THAW FOR YEAR		47.2	55.5	60.7				
CONTROL CROSS SECTIONS	1M	--	62.3	63.7	--	+ 2.2 %	--	+ 0.7 %
	3	--	61.6	61.9	--	+ 0.5 %	--	
	6	--	52.0	52.4	--	+ 1.6 %	--	
	9	--	52.0	52.4	--	+ 0.8 %	--	
	14	--	54.6	53.5	--	- 2.0 %	--	
	20	--	55.6	56.3	--	+ 1.3 %	--	
AVERAGE THAW FOR YEAR		--	57.1	57.5				
DEGREE DAYS OF THAWING		3575.	3567.	3576.				

1976 there had been a total of 3575 degree days of thawing. On 20 September 1977, there had been 3567 degree days of thawing. And on 26 September 1978 there had been 3576 degree days of thawing.

One full thaw season (1977) following the summer spill, significant increases occurred in the average thaw depth in the winter and summer spills as compared to 1976, 21.2% and 17.5% respectively. Data from the control plot is unavailable for 1976. We assume there was little or no change in depth of thaw from 1976 to 1977 in the control plot. This is based on the lack of change from 1977 to 1978 and the equivalent degree days of thawing for 1976, 1977 and 1978.

After the second full thaw season, additional smaller increases in the thaw depths in the winter and summer plots occurred. Thaw depths in the summer plot increased by 9.4% over the previous year and in the winter plot, with its greater surface-oiled area, by 13.2%. Although others have reported little increase in the active layer beneath crude oil spills (Hutchinson and Freedman 1975; Freedman and Hutchinson 1976), measurements of the active layers in this study show an increasing active layer several years after the initial spills.

Two full years of this data are insufficient to predict the long term effects of the oil on permafrost. The thawing trend underway may stabilize or even reverse itself, for example, if vegetation starts to re-establish on the spill areas. Continuation of the present trend could result in catastrophic thermokarsting. However, stabilization of thaw seems most likely as long as the organic mat above the mineral soil is not destroyed.

Compositional Changes

The Prudhoe Bay crude oil used in the study was characterized by a number of chemical methods in order to assess changes due to natural weathering on slope. These included fractionation with respect to major classes of components by column chromatography and further characterization of each fraction by gas chromatography and infrared spectroscopy. Some of the pertinent data obtained are summarized in Table 2. A gas chromatogram of the original oil is shown in Figure 12.

The major processes thought to contribute to weathering of oil after a terrestrial spill are evaporation of volatiles, solubility and translocation of water-soluble components, and microbiological degradation. Of these, the loss of volatiles was found to make the largest initial impact. Photochemical degradation, many times the controlling factor in degradation of oil in marine spills, is negligible in terrestrial systems where the amount of oil exposed to sunlight is small.

TABLE 2. INITIAL CHARACTERISTICS OF PRUDHOE BAY CRUDE OIL USED IN STUDY

Specific Gravity	0.89
% Sulfur	1.0%
% Volatiles (C_1-C_8)	8%
(C_9-C_{15})	15%
Major Components of Residue (C_{15}-C_{40})	
Alkanes	33%
Aromatics	30%
Asphaltenes	22%
Soluble NSO	11%
Insoluble NSO	4%
Pristane/n-C_{17} Ratio	0.65
pH	7.2
Alkane/Aromatic Ratio	1.12

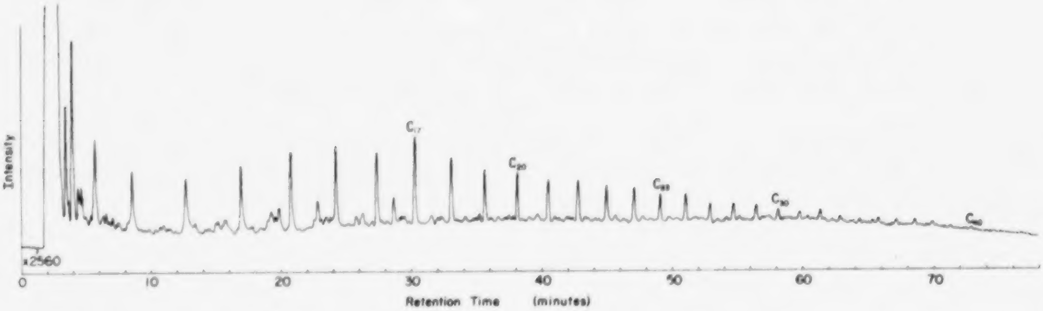


Figure 12. Gas chromatogram of the original oil used for the Caribou-Poker spills obtained on a Dexsil Scot column (50 ft x 0.02 in., temperature programmed from 75° to 350° at 4°C/min.).

Samples of the original oil, oil from pools in the spill area, and oily and control soil, were analyzed by head space gas chromatography to characterize changes in the most volatile fraction (C_1 through C_8). Changes in the presence of these components in the equilibrium headspace at a given temperature are a direct reflection of their change in concentration in the liquid (oil) phase. Results of these analyses are shown in Figure 13 and Table 3. These chromatograms, obtained for samples collected from a surface pool of oil on the winter spill site, show a rapid loss of methane and ethane (C_1 and C_2) in the first few hours. Components in the C_3-C_8 range (propane-octanes) declined through the first day with reduction by a factor of four in the first two months. Only small amounts of these components (C_3-C_8) were observable in the five-month sample. The retention of these low molecular weight volatile substances for some time after spillage undoubtedly maintained greater fluidity in the oil resulting in substantial remobilization of the oil downslope at spring breakup. This pool of oil on the winter slope was sampled again and analyzed for volatiles 17 months after spillage. No detectable components in the C_1 - C_8 range were found at that time. Estimates of the percent of volatiles in the C_1 - C_8 range lost with time, based on these chromatograms, are presented in Table 3. Two months after spillage, over 10% of this volatile material was still present in the oil located on the surface of the soil.

Analyses were also obtained for the oil in the soil profile from the control and spill plots. No volatiles in the C_1 - C_8 range were ever detected in any of the control soils. Results from the winter spill soils indicate a slower loss of volatiles when compared with the surface pool of oil. Results from this analysis for soils collected 17 months after spillage are presented in Figure 14 and Table 3. Significant amounts of volatiles, in the C_5 - C_8 range, were still present in the mineral and organic soils at that time. No detectable volatiles were present in the surface moss after 17 months however.

Analysis of volatiles in soil from the summer spill plot were also obtained. Initially the volatiles were present in much higher concentration than in the winter spill since fresh oil penetrated the soil at the time of the summer spill. In contrast, oil in the winter spill remained on the surface for several months until warmer temperatures allowed it to penetrate into the organic and mineral soil. The loss rate of volatiles in the summer spill soils, in general, was greater than that found for the winter spill. Thus after 12 months exposure in the summer spill, there were lower concentrations of volatiles (Fig. 15 and Table 3) than in the winter spill plot after 17 months (Fig. 14), for both the organic and mineral soils.

These results, showing substantial retention of volatiles well after the spill, conflict with those reported for arctic tundra soils at Barrow, Alaska (Sexstone and Atlas, 1977). At Barrow complete loss of

Table 3. LOSS OF VOLATILES* FROM SPILL PLOTS.

	Percentage of volatiles* evaporated			
Time after spillage	Surface Oil	Oil in Moss	Oil in organic soil	Oil in mineral soil
		Winter Spill Plot		
Original Oil	0%			
1 Hour	0.8%			
1 Day	34.6%			
2 Months	88.6%			
5 Months	99.3%	98.2%	71.3%	75.2%
17 Months	100%	100%	96.6%	94.9%
		Summer Spill Plot		
Original Oil	0%			
1 Hour	1.7%			
2 Hour	17.6%			
3 Hour	26.2%			
4 Hour	17.2%			
22 Hour	46.6%			
2 Days	64.6%			
3 Days	59.0%			
2 Months		89.2%	89.8%	
8-1/2 Months		99.9%	99.1%	98.3%
12 Months		99.9%	99.0%	99.9%
12-1/2 Months				

* Volatiles are considered the components in the C_1 - C_8 range and account for about 8% of the oil by weights.

the volatile fraction ($<C_{10}$) was reported in the first 24 hours. This difference may be explained by differing methods of oil application or may be due to the location of the oil in the top 2 cm of the soil at Barrow. Oil at Barrow was applied evenly through a perforated plate, resulting in a thin film in closer contact with the atmosphere. Their results may be questionable, however, due to the use of a solvent extraction method not well suited for analysis of compounds in the $<C_{10}$ range (MacKay and Shiu, 1976).

In another study at Prudhoe Bay, retention of C_9 and C_{10} components in the soil, a year after spillage, has been reported (Sexstone et al., 1978). While C_1 - C_8 components were not found in their analysis, soil samples collected from these spill plots and analyzed in our laboratory did reveal low levels of C_6 - C_8 material, 12 months after spillage (Fig. 16).

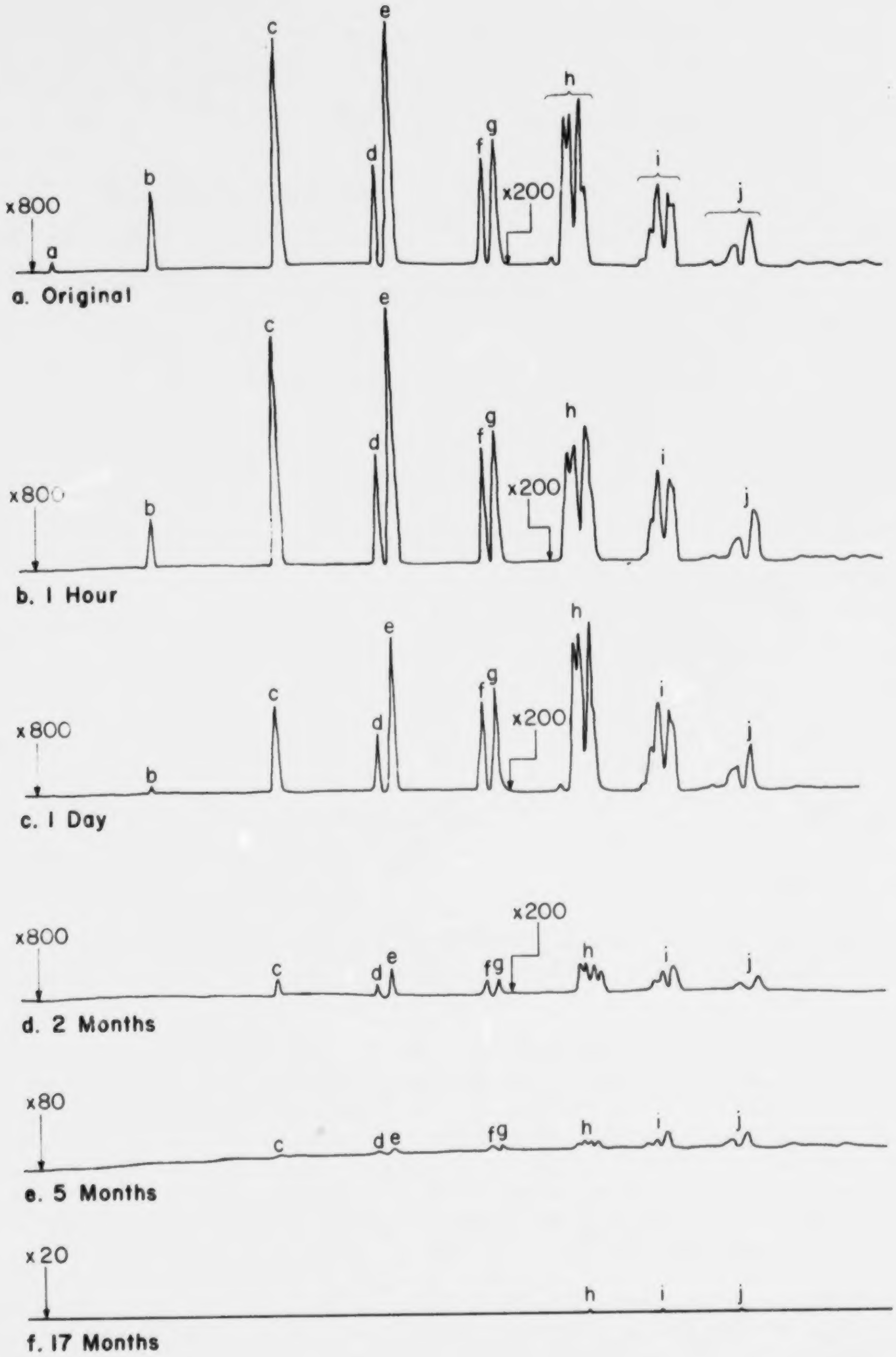


Figure 13. Headspace chromatograms of oil taken from a pool on the winter spill plot. (5% Durapak 400 on Porasil C, 9 ft x 1/8 in., temperature programmed from -20° to 150°C at 10°/min).

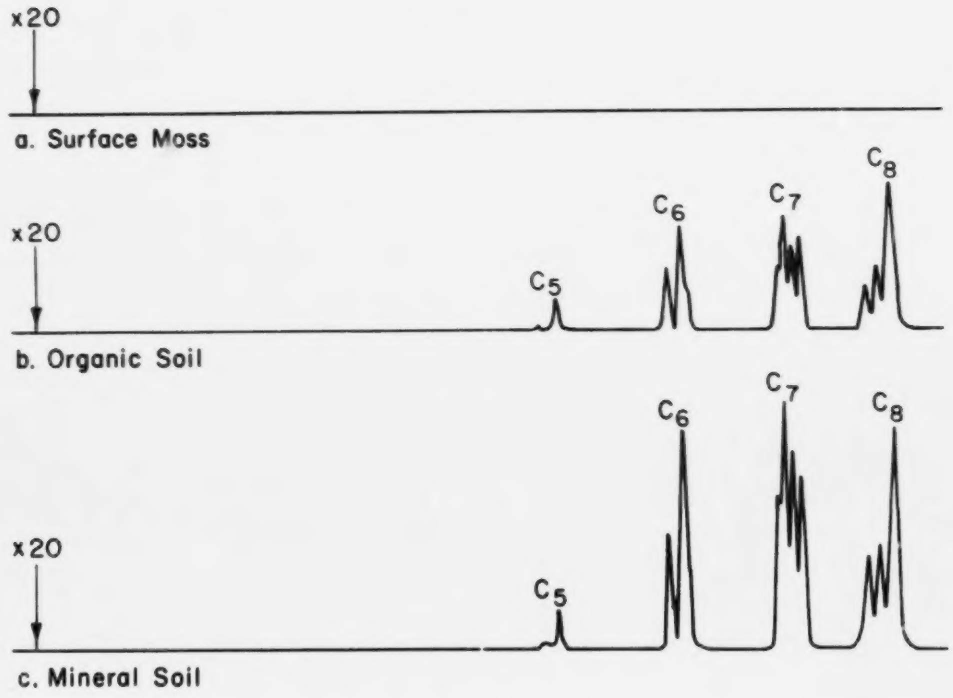
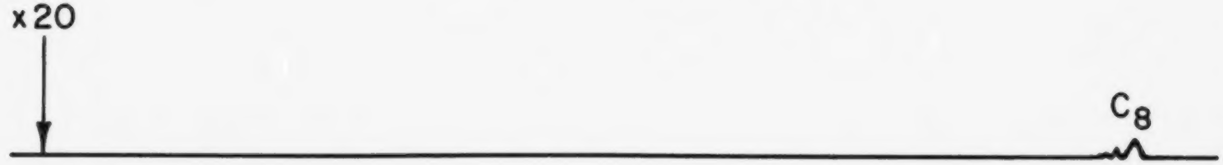
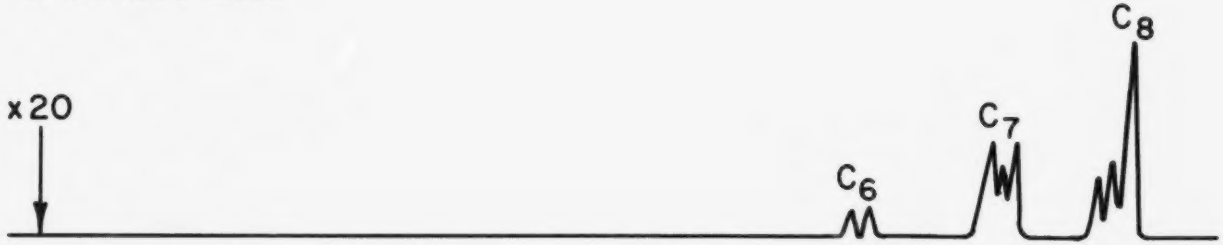


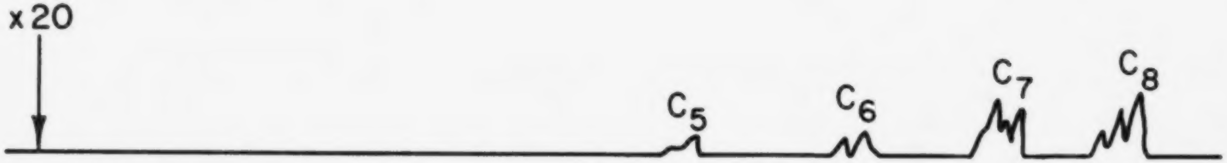
Figure 14. Headspace chromatograms of oily soil from winter spill plot, 17 months after spillage (GC conditions were identical to those given for Figure 13).



a. Surface Moss



b. Organic Soil



c. Mineral Soil

Figure 15. Headspace chromatograms of oily soil from the summer spill plot, 12 months after spillage. (GC conditions were identical to those given for Figure 13).

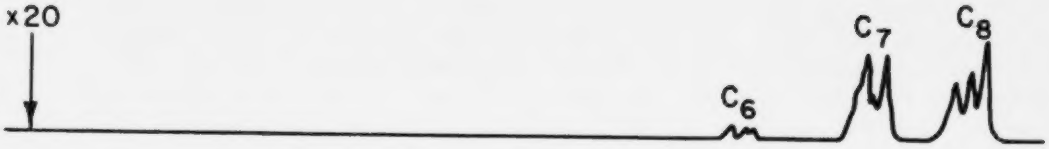


Figure 16. Headspace chromatogram of oily soil taken from the Prudhoe Bay spill site, 12 months after spillage. (GC conditions were identical to those given for Figure 13).

Thus it appears that volatiles are retained much longer in the soil profile than originally believed. Some of these volatile substances, particularly the lower molecular weight aromatics (i.e. benzene, toluene, xylenes), are thought to be very toxic to plants and microorganisms. Their continued presence well after the spill may be biologically significant.

Runoff is undoubtedly responsible for some translocation of water-soluble material out of the spill areas. Attempts at measuring the amounts of materials removed by this mechanism were unsuccessful. Water samples collected at locations just downslope of visible oil movement were analyzed by a simplified stripping method developed by Leggett (1979). No detectable quantities ($>10^{-9}$ g/ml) of individual components were found in any sample. An attempt at utilizing total organic carbon analysis to look at the total dissolved material was also unsuccessful due to large concentrations of organic carbon (>50 mg/ℓ) in the natural soil solution of this region.

In general, the most water-soluble components of the oil are also the most volatile. This was demonstrated in a study of the aqueous solubility of Prudhoe Bay crude oil (MacKay and Shiu, 1976). A decrease in solubility from over 29 mg/l for fresh oil to less than 0.06 mg/l for oil in which the volatiles had been removed by evaporation was found. The loss rate of equally volatile substances, which differ widely in solubility, can be used to qualitatively assess the importance of this mechanism relative to volatilization. Losses of two very soluble components of the oil, benzene and toluene, were found to be similar to the losses of alkanes of equal volatility but much lower solubility. Thus, at least for the lower molecular weight oil fraction, the contribution of solubility to loss rate is less significant than volatilization.

Results obtained from silica gel-alumina chromatography (to be presented later) indicate that compositional changes of relatively water-soluble material versus highly insoluble material in the oil extracted from soil samples are insignificant. These results are consistent with information reported by Raymond et al. (1976) in a study in a more temperate climate where no loss of material was observed via runoff or leaching in oiled field plots. They also agree with results obtained for spills directly onto fresh water (Phillips and Groseva, 1977) and salt water (McAuliffe, 1977) where evaporation was shown to be the predominant process for short-term changes in oil composition after spills.

Several types of microorganisms, including bacteria, actinomycetes, yeasts, and fungi have been shown to be capable of degrading many of the organic components of crude oils (Davis, 1967). The total amount of oil present on the site at one time cannot be directly measured without destroying the site. Hence, it is impossible to determine the change due to microbial activity in the amount of oil present. Laboratory studies, however, have shown preferential decomposition of certain fractions of the oil. Bailey et al. (1973) and Katov et al. (1971) have shown that the alkane fraction (saturates) decompose more rapidly than other components. Shorter chain n-alkanes (straight chains) were found to decompose fastest of all. Isoprenoid alkanes (branched chained species), such as pristane, have been shown to be more resistant to microbiological degradation (Jobson et al., 1972).

Aromatic components tend to degrade at the next fastest rate to the alkane fraction with degradation of asphaltene and NSO material being very slow. An increase in asphaltene material as a by-product of decomposition of more degradable material has even been reported (Bailey et al., 1973). Assessment of the extent of microbial degradation is thus possible, in principle, by analysis of oil compositional changes with time.

The method chosen to investigate these changes was fractionation using silica gel-alumina column chromatography. This procedure subdivides the residue obtained by chloroform extraction of the oily soils into its major organic components: alkanes, aromatics, asphaltenes and NSOs. The ratio of alkane to aromatic material was chosen as an indicator of compositional change with time. These ratios obtained from oil extracts from the soil from July, 1976 through June, 1978 are presented in Table 4. The Prudhoe Bay oil used for the spill has an initial 1.12 alkane/aromatic ratio.

TABLE 4. ALKANE/AROMATIC AND PRISTANE/n-C_{17} RATIOS* FOR TOPPED** SOIL EXTRACTS (CARIBOU-POKER CREEK AREA SPILLS)

Sampling Date	Winter Mineral Soil (C_2)		Winter Organic Soil (O_2A_1)		Winter Moss	
	a	b	a	b	a	b
7/15/76	1.03	0.59	1.10	--	1.22	0.57
9/17/76	1.29	0.56	1.27	0.58	1.23	0.61
3/29/77	--	--	1.05	0.53	1.25	0.58
4/5/77	1.08	0.58	1.29	--	1.28	0.62
7/13/77	1.04	0.53	1.17	0.55	1.01	0.67
8/1/77 ***	1.17	0.50	1.09	0.56	--	--
1/5/78	--	--	--	--	0.84	0.71
6/27/78***	1.11	0.54	0.98	0.55	--	--

	Summer Mineral Soil (C_2)		Summer Organic Soil (O_2A_1)		Summer Moss	
	a	b	a	b	a	b
7/15/76	1.12	0.61	1.12	0.59	1.03	0.59
9/17/76	1.05	0.56	1.05	0.64	1.12	0.58
3/29/77	--	--	1.36	0.56	0.99	0.56
4/5/77	0.98	0.54	1.00	0.55	--	--
7/13/77	--	--	1.04	0.51	1.02	0.63
8/1/77***	0.77	0.68	1.14	0.60	--	--
1/5/78	--	--	0.87	0.60	0.94	0.76
6/27/78***	1.10	0.71	0.96	0.58	--	--

a Alkane/aromatic ratio (The measured precision of this ratio is ±0.03).

b Pristane/n-C_{17} ratio (The precision of this determination is estimated to be ±0.03).

* Original topped Prudhoe Bay oil had ratios of 1.12 for alkane/aromatic and 0.65 for Pristane/n-C_{17}.

** Topped extracts are those in which the volatile fraction ($<C_{15}$) has been removed by evaporation.

*** These samples were collected as described in the soil microbiological methodology. All others were collected as described in the oily soil characterization section.

A second method based upon gas chromatographic analysis of the alkane fraction was developed to follow the ratio of pristane, an isoprenoid alkane, to n-C_{17}. Since these species have similar volatility and very low solubility, a change in their ratio should be indicative of microbiological degradation. The results of these analyses are also presented in Table 4. Initially Prudhoe oil had a 0.65 pristane/n-C_{17} ratio.

The results from both of these determinations indicate very little or no compositional change with time through the first two years with respect to these parameters. The only possible exception could be the oil in the summer mineral soil, where some increase in pristane/n-C_{17} ratio was observed in the 8/1/77 and 6/27/78 samples. Samples taken July 1978 (to be discussed later, see Table 6) do not confirm this trend. Oil in the moss may also show some small change. Additional sampling in the next several years will be required to confirm that observable compositional change due to microbial activity is occurring.

If these parameters are accepted as a measure of the effect of microbial activity, little compositional change due to microbial activity can be inferred thus far. If, however, microbial degradation is occurring in a more non-specific fashion relative to the various oil components, these parameters might not be useful to discriminate this type of natural weathering. Some recent evidence does suggest that degradation in field conditions can be less preferential (Raymond et al., 1976; Horowitz and Atlas, 1977) than has been observed in laboratory studies.

In order to assess if these parameters are useful in detecting changes due to microbial activity, several other oil spill plots in Alaska, some dating back to 1970, were sampled and analyzed in a manner similar to the Caribou-Poker spills. Chromatograms for oil from some of these spills, as well as from initial Prudhoe crude, are shown in Figure 17. The results of these analyses are shown in Table 5.

The results from the 1970 Barrow spills show considerable changes in the pristane/n-C_{17} ratios with the value for the plot 313 showing nearly a tenfold increase to 5.1. The other Barrow spills are also elevated above that found in the Caribou-Poker spills. The chromatograms from which these values were obtained for Barrow plots 313 and 311 are presented in Figure 17. The chromatograms for Barrow plot 313, in particular, show a drastic change from original Prudhoe Crude Oil, with the normal alkanes being much reduced relative to branched chain material. The alkane/aromatic ratio shows much less change relative to the values typically found in the Caribou-Poker spills. Barrow sample 313 did show a decrease in alkane/aromatic ratio to about 0.84 from values generally greater than 1.0 for the Caribou-Poker sites. These results indicate that the pristane/n-C_{17} ratio may be a useful tool to assess the effect of microbial activity on the oil composition in the soil. The alkane/aromatic ratio was found to be much less diagnostic.

TABLE 5. ALKANE/AROMATIC AND PRISTANE/n-C_{17} RATIOS FOR SAMPLES FROM OTHER ALASKAN SPILLS

SAMPLE (Spill Date)	Sample Date	Alkane/Aromatic Ratio	Pristane/n-C_{17} Ratio	% Oil in Soil
Barrow 313 (1970)	July 1977	0.84	5.1	4.4%
Barrow 311 (1970)	July 1977	1.00	0.80	-
Barrow 152D(1971)	July 1977	0.96	0.81	13.3%
Fox (1971)	July 1976	0.92	0.63	249%
Prudhoe Oil used For Prudhoe Spill (July 1976)		0.96	0.64	-
Prudhoe 2CR (1976)	July 1977	1.06	0.55	7.7%
Prudhoe 5CR (1976)	July 1977	0.97	0.56	11.6%
Caribou-Poker Winter Spill (Feb. 1976)	1976-1978	(see Table 4)	(see Table 4)	Moss 137-267% Organic 7.3-190% Mineral 3.0-9.8%
Caribou-Poker Summer Spill (July 1976)	1976-1978	(see Table 4)	(see Table 3)	Moss 69-207% Organic 9-311% Mineral 1.5-3.4%

Soil samples were also obtained from the Fox spill site. This site is quite similar to the Caribou-Poker area, and is located on a subarctic permafrost location. The amount of oil present in the soil at Fox is very high relative to the Barrow site and again similar to the Caribou-Poker area. A chromatogram of the alkane fraction of the oil from this site is given in Figure 17. Even after five years, there appears to be no evidence of significant microbial decay at this location (Table 5).

The oil extracted from soil at the Prudhoe Bay spills also shows little compositional change relative to the original oil after one year (Fig. 17 and Table 5). The percent oil in the soil at this site is similar to the Barrow spill areas and much lower than found in Fox or Caribou-Poker. This lower amount should make a small change in composition due to microbial activity much more observable in the future.

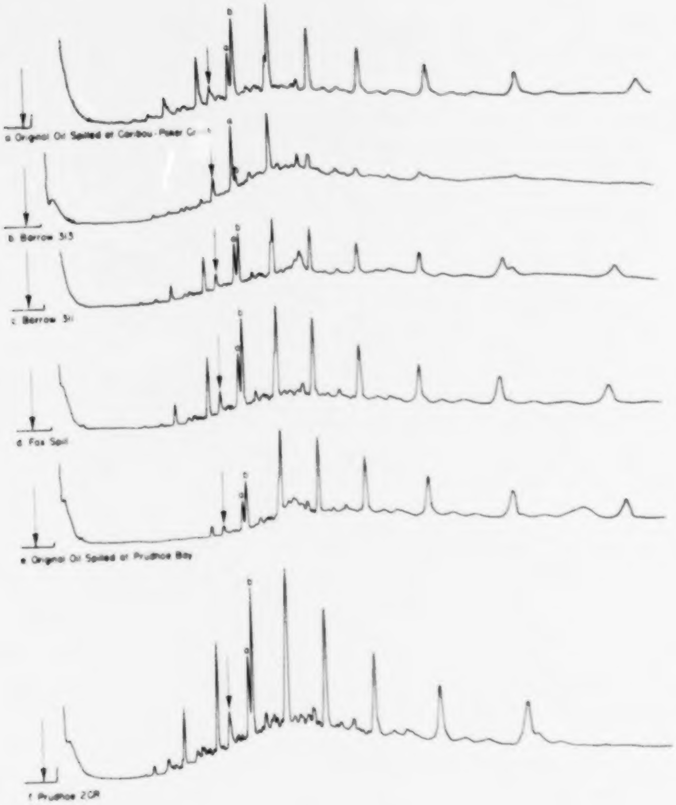


Figure 17. Chromatograms of oil extracted from soil taken from several Alaskan oil spill sites (Apiezon L SCOT column, 50 ft x 0.02 in., temperature programmed from 175° to 250° at 4°/min.).

The analyses reported thus far for the Caribou-Poker spills were taken in the upper, heavily impacted areas of both plots. Additional samples were collected in July 1978 to determine the vertical distribution of oil at various downslope locations in both plots (Fig. 10a-f) and to assess if different amounts of weathering were occurring at other locations.

The amount of oil in the various soil horizons of the winter and summer spill plots was found to vary drastically from location to location (Table 6). In general, the moss and upper organic soil has the greatest amount of oil in the upslope portion of the winter spill plot with amounts in the moss varying from 137% to 267% of the dry weight of the soil. Amounts of oil in the organic and mineral soil of the winter plot vary from 7.3% to 190% and 3.0% to 9.8%, respectively.

TABLE 6. ANALYSES OF SAMPLES COLLECTED IN JULY 1978

SAMPLES		% Oil	Pris/n-C_{17}	% Alkanes	Alk/Arom
S 4-5, 8-9	(MOSS)	183.0	0.71	31.8	0.77
S 1-2, 8-9	(MOSS)	43.1	0.54	-	-
S 7, 34	(MOSS)	135.0	0.67	31.9	0.94
S 4-5, 8-9	(ORGANIC)	311.0	0.56	36.8	1.05
S 7, 34	(ORGANIC)	131.0	0.54	32.0	0.81
S 1-2, 8-9	(ORGANIC)	57.9		32.9	0.97
S 1-2, 8-9	(MIN)	2.4		22.1	0.89
S 8-9, 8-9	(MIN)	22.0		30.0	0.96
W 8-9, 11-12	(MOSS)	246.0	0.61	32.4	0.93
W 8-9, 11-12	(ORGANIC)	91.3	0.54	33.0	0.94
W 7, 34	(ORGANIC)	30.8		30.6	1.01
W 8-9, 11-12	(MIN)			33.5	0.98
S ORGANIC	June 1978	21.1	0.58	30.9	0.96
S MINERAL	" "	1.76	0.71	26.6	1.1
W ORGANIC	" "	16.8	0.55	33.0	0.98
W MINERAL	" "	3.89	0.54	31.0	1.06

In the upper portion of the summer spill area, the largest amount of oil is in the organic soil and lower moss. Amounts of oil in the moss vary from 69% to 207%,and in the organic layer vary from 9% to 311% The mineral soil, on the other hand, contained much less oil varying from 1.5% to 3.4%.

In the lower sections of both spill plots, the location of oil in the profile is considerably different. In the winter plot, the oil resided in a narrow band within the organic soil with an oil content in this region of about 30% (Fig. 12). In the lower portion of the summer plot, the oil was present throughout the organic layer with small amounts present in the lower moss and upper mineral layers as well. The moss and organic soil contained in excess of 100% of oil in this region.

Analysis of the residual oil extracted from soil samples collected in July 1978 at various locations in the two spill plots showed little difference in composition (Table 5). Thus there does not seem to be a drastic difference in the amount of microbial or other types of natural weathering in various areas within the plots.

Microbiological Responses

A preliminary examination of the data suggests that the microbial populations and their activities showed similar response trends at both incubation temperatures of 4°C and 20°C (20°C incubation results are presented in appendix C). Also, respiratory activity and some microbial populations were stimulated to a greater extent at 4°C than at 20° C. Thus only the 4°C data is presented here since 4°C more closely approximates the soil temperature in the research watershed areas where our test plots are located (Appendix Table A-1). The dilution plate count method and MPN enumeration procedures were used in this study. Despite their limitations these methods are sensitive to differences in microbial counts among the test plots or within a plot over a period of time. Relative changes in numbers of microorganisms are more important than absolute numbers.

The effect of oil application on heterotrophic bacterial counts is shown in Tables 7, 8 and 9. In 1976, beginning immediately after the spill in the winter plot, the numbers of heterotrophic bacteria were increased relative to the control plot, and the increased counts persisted into September. In the summer plot, after an initial depression of the heterotrophic bacterial counts, there was a continual increase in numbers with the highest counts occurring in September. These increased bacterial counts in the oiled plots persisted through 1977 (Table 8) and 1978 (Table 9). The increases in bacterial numbers in both oiled plots were significant relative to the control plot indicating a stimulatory effect of the spill on the heterotrophic bacterial population, the extent of stimulation being greater in the summer. Our findings on the response of heterotrophic bacterial populations to oil agree with those of Sexstone and Atlas (1977) and Loynachan (1978).

TABLE 7. 1976 HETEROTROPHIC BACTERIAL COUNTS (x 10^6/g soil) IN OILED AND UNOILED (CONTROL) PLOTS

Sampling time	Control plot	Winter plot	Summer plot
		A_1 Horizon	
February[a]	2.8	5.4**	-
June	6.8	120**	-
July[b]	3.9	430**	2.8*cc
August	2.4	170**	340**cc
September	0.7	210**	640**cc
		C_2 Horizon	
February[a]	1.2	1.8*	-
June	2.2	2.1	-
July[b]	0.9	41**	0.6**cc
August	1.4	14**	25**cc
September	0.3	12**	210**cc

a Within 24 hours after winter oil spill.
b Within 24 hours after summer oil spill.
* Significantly different from the control at 5% level.
** Significantly different from the control at 1% level.
cc Significantly different from the winter at 1% level.

TABLE 8. 1977 HETEROTROPHIC BACTERIAL COUNTS (x 10^6/g soil) IN OILED AND UNOILED (CONTROL) PLOTS.

Sampling time	Control plot	Winter plot	Summer plot
		A_1 Horizon	
June	7.7	540**	1400**cc
July	1.9	240**	900**cc
August	1.0	99**	460**cc
		C_2 Horizon	
June	1.1	36**	210**cc
July	0.3	63**	110**cc
August	0.4	40**	77**cc

** Significantly different from the control at 1% level.
cc Significantly different from the winter at 1% level.

TABLE 9. 1978 HETEROTROPHIC BACTERIAL COUNTS (x 10^6/g soil) IN OILED AND UNOILED (CONTROL) PLOTS.

Sampling time	Control plot	Winter plot	Summer plot
		A_1 Horizon	
June	2.9	220**	480**cc
July	1.6	190**	150**c
		C_2 Horizon	
June	0.8	58**	43**cc
July	0.4	42**	21**cc

** Significantly different from the control at 1% level.
c Significantly different from the winter at 5% level.
cc Significantly different from the winter at 1% level.

Numbers of filamentous fungal propagules (Table 10) were reduced in both soil horizons immediately following the winter spill and remained so throughout the growing season. The immediate impact of the summer spill was to reduce fungal counts in the A_1 horizon, but by September, fungal counts were five times higher than in the control. In the C_2 horizon there was an overall increase in counts with an apparent reduction occurring in August. However, in 1977 (Table 11) and 1978 (Table 12), fungal propagules were significantly reduced in both soil horizons of the oiled plots. The extent of oil inhibition on the filamentous fungal population was greater in the winter plot than in the summer plot. The inhibitory effect of oil spills on fungal populations (investigated by the dilution plate method) were similarly observed by other workers (Miller _et al_., 1978; Antibus and Linkins, 1978), who used direct counting techniques to estimate the fungal populations. Warcup (1967) suggested that the dilution plate method may not necessarily correlate with fungal biomass or activity. However, according to Griffiths and Siddiqi (1961) and Montégut (1960), the dilution plate counts can be used as an indication of the fungal populations and their activities.

In 1976, yeast counts in both oiled plots were significantly increased above their initial levels and relative to those in the control plot (Table 13). These increases in counts relative to the control persisted in 1977 (Table 14) and 1978 (Table 15). Numbers in the summer plot were consistently higher than those in the winter plot during the three consecutive growing seasons after the oil spills. The significant increases suggest an enhancement of the yeast population resulting from the oil applications. A similar response was noted by Scarborough and Flanagan (1973) and Campbell _et al_ (1973).

TABLE 10. 1976 FILAMENTOUS FUNGAL PROPAGULE COUNTS (x 10^4/g soil) IN OILED AND UNOILED (CONTROL) PLOTS

Sampling time	Control plot	Winter plot	Summer plot
		A_1 Horizon	
February[a]	130	92*	-
June	120	14**	-
July[b]	120	18**	49**c
August	34	23**	31*
September	24	10**	100**cc
		C_2 Horizon	
February[a]	19	4**	-
June	24	0.7**	-
July[b]	13	8**	17**cc
August	14	4**	5**
September	5	2**	7*cc

a Within 24 hours after winter oil spill.
b Within 24 hours after summer oil spill.
* Significantly different from the control at 5% level.
** Significantly different from the control at 1% level.
c Significantly different from the winter at 5% level.
cc Significantly different from the winter at 1% level.

TABLE 11. 1977 FILAMENTOUS FUNGAL PROPAGULE COUNTS (x 10^4/g soil) IN OILED AND UNOILED (CONTROL) PLOTS

Sampling time	Control plot	Winter plot	Summer plot
		A_1 Horizon	
June	110	27**	43**
July	64	13**	34**cc
August	60	1.4**	18**cc
		C_2 Horizon	
June	30	2.1**	15**cc
July	4.8	1.3**	5.1cc
August	11	0.6**	5.4**cc

* Significantly different from the control at 5% level.
** Significantly different from the control at 1% level.
cc Significantly different from the winter at 1% level.

TABLE 12. 1978 FILAMENTOUS FUNGAL PROPAGULE COUNTS (x 10^4/g soil) IN OILED AND UNOILED (CONTROL) PLOTS

Sampling time	Control plot	Winter plot	Summer plot
		A_1 Horizon	
June	78	19**	56**cc
July	62	18**	43**cc
		C_2 Horizon	
June	12	5.6**	7.4**cc
July	19	6.7**	12 **cc

** Significantly different from the control at 1% level.
cc Significantly different from the winter at 1% level.

TABLE 13. 1976 YEAST COUNTS* (x 10^5/g soil) IN OILED AND UNOILED (CONTROL) PLOTS

Sampling time	Control plot	Winter plot	Summer plot
		A_1 Horizon	
February[a]	1.9	4.7**	-
June	3.4	320 **	-
July[b]	1.3	31 **	3.1**cc
August	0.9	44 **	640 **cc
September	1.1	12 **	650 **cc
		C_2 Horizon	
February[a]	0.9	1.0	-
June	0.8	2.9**	-
July[b]	0.5	5.0**	0.7cc
August	0.4	7.4**	64 **cc
September	0.2	3.3**	290 **cc

a Within 24 hours after winter oil spill.
b Within 24 hours after summer oil spill.
** Significantly different from the control at 1% level.
cc Significantly different from the winter at 1% level.

TABLE 14. 1977 YEAST COUNTS (x 10^5/g soil)
IN OILED AND UNOILED (CONTROL) PLOTS

Sampling time	Control plot	Winter plot	Summer plot
		A_1 Horizon	
June	2.0	32 **	140 **cc
July	3.2	14 **	64 **cc
August	3.2	6.3**	54 **cc
		C_2 Horizon	
June	.19	1.3**	23 **cc
July	.16	2.7**	3.6**cc
August	.20	1.3**	3.6**cc

** Significantly different from the control at 1% level.
cc significantly different from the winter at 1% level.

TABLE 15. 1978 YEAST COUNTS (x 10^5/g soil)
IN OILED AND UNOILED (CONTROL) PLOTS

Sampling time	Control plot	Winter plot	Summer plot
		A_1 Horizon	
June	2.4	38**	300**cc
July	3.3	23**	95**cc
		C_2 Horizon	
June	0.8	10**	18**cc
July	0.6	11**	24**cc

** Significantly different from the control at 1% level.
cc Significantly different from the winter at 1% level.

The response of proteolytic bacteria was monitored only during 1976. In both oiled plots, there were significant increases in counts above their initial levels and relative to the control (Table 16). This indicates a general stimulation of the proteolytic bacterial population due to the oil applications, with the extent of stimulation being significantly greater in the summer plot compared to the winter plot.

TABLE 16. 1976 COUNTS (x 10^5/g soil) OF PROTEOLYTIC BACTERIA IN OILED AND UNOILED (CONTROL) PLOTS

Sampling time	Control plot	Winter plot	Summer plot
		A_1 Horizon	
February[a]	1.9	2.6	-
June	1.7	3.1**	-
July[b]	1.0	100 **	1.1 cc
August	0.9	57 **	1000 **cc
September	1.0	95 **	1100
		C_2 Horizon	
February[a]	0.6	0.6	-
June	0.4	6.3	-
July[b]	0.7	23 **	0.2**cc
August	0.2	5.1**	110 **cc
September	0.2	5.3**	370 **cc

[a] Within 24 hours after winter oil spill.
[b] Within 24 hours after summer oil spill.
** Significantly different from the control at 1% level.
cc Significantly different from the winter at 1% level.

The effect of the oil spills on anaerobic bacterial counts were followed only in the months of July and September in 1976. In the winter plot, significant increases in counts relative to the control plot occurred during both months; in the summer plot, significant increases occurred in September (Table 17). These results indicate an enhancement of anaerobic bacterial growth during the first growing season after the spills. Loynachan (1978) reported similar findings on the effect of oil on anaerobic bacteria.

The denitrifying bacterial population consistently showed significant increases in both winter and summer plots relative to the control plot (Figs. 18, 19 and 20). The significant increases in the denitrifying bacterial numbers in the oiled plots suggest an increase in denitrification potential and indicate a stimulatory effect of oil. This is in agreement with findings of Lindholm and Norrell (1973). However, the stimulatory effect of oil on the denitrifying bacterial population seems to diminish with time. A decrease in population levels in both oiled plots is apparent in 1978 relative to 1977 or 1976 levels, with 1977 counts being lower than 1976 counts.

TABLE 17. 1976 COUNTS (x 10^5/g soil) OF ANAEROBIC BACTERIA IN OILED AND UNOILED (CONTROL) PLOTS.

Sampling time	Control plot	Winter plot	Summer plot
		A_1 Horizon	
July[a]	5.1	54 *	3.6cc
September	3.0	36 **	42 **
		C_2 Horizon	
July[a]	2.0	7.7 **	1.6cc
September	2.0	4.5 **	10 **cc

a Within 24 hours after summer oil spill.
* Significantly different from the control at the 5% level.
** Significantly different from the control at the 1% level.
cc Significantly different from the winter at the 1% level.

Microbial populations capable of utilizing cellulose as a sole carbon source were monitored only in 1976. Cellulose-utilizing bacterial numbers (Figure 21) increased in the winter plot relative to the control. In the summer plot, this population was initially unaffected, but by September significant increases in counts over the control occurred. On the other hand, numbers of cellulose-utilizing fungi (Figure 22) remained comparable to the control in both oiled plots. The significant increase in cellulose-utilizing bacteria suggests stimulatory effects of oil on this population.

In 1976 oil-utilizing yeasts and bacteria (Figures 23 and 24 respectively) in the winter plot were significantly increased relative to the control. In the summer plot, counts of these oil-utilizing populations were initially comparable to the control but by September increases had occurred. The significant increases in the oil-utilizing bacterial population in both oiled plots continued through 1978 (Figure 25). However for the oil-utilizing yeasts, significant increases relative to the control persisted through 1978 only in the A_1 horizon of the summer plot (Figure 26). In the C_2 horizon of both oiled plots, counts of this yeast population were significantly higher than the control only in 1978.

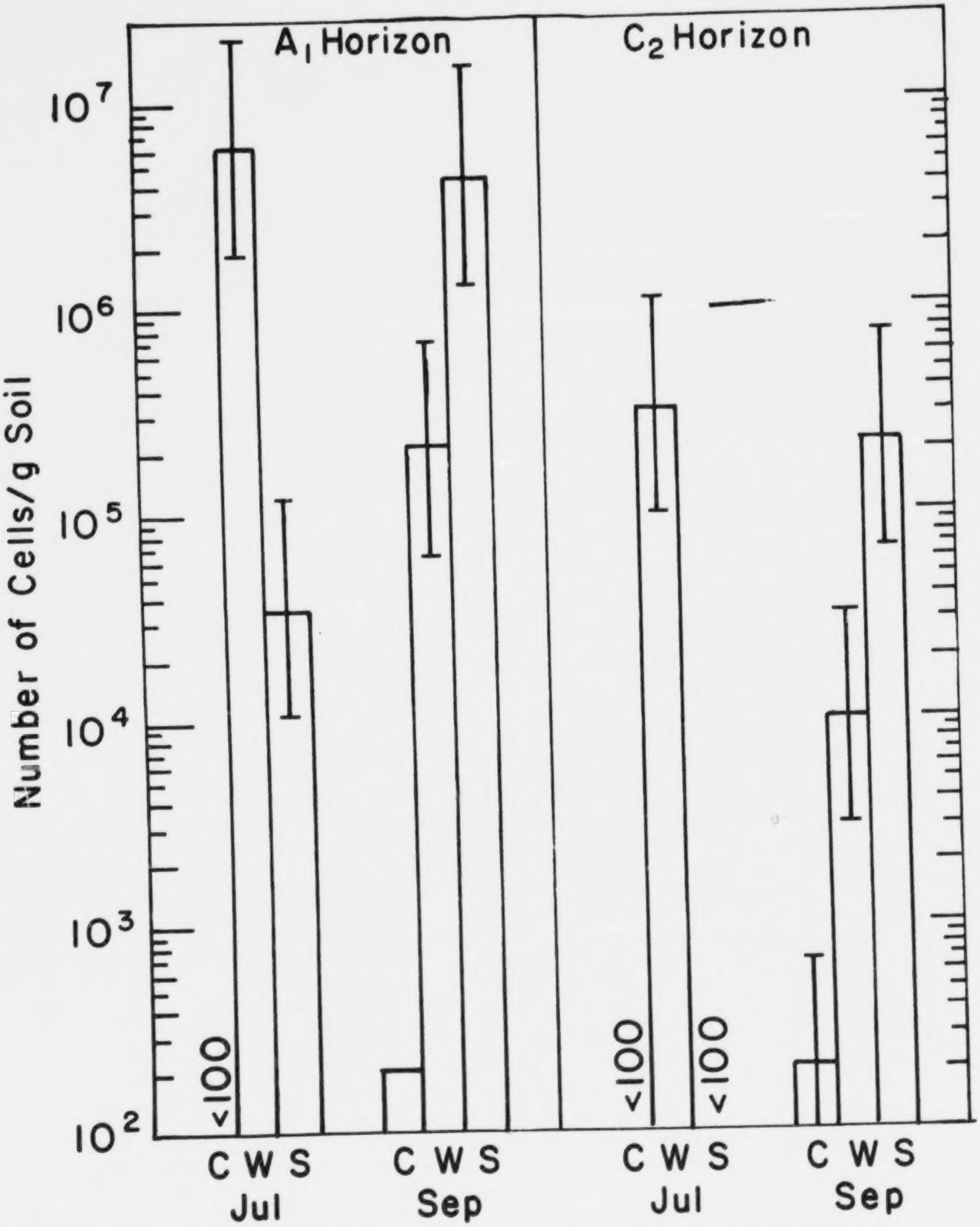


Figure 18. 1976 MPN counts of denitrifying bacteria in oiled and unoiled (control) plots. C = Control Plot; W = Winter Plot; S = Summer Plot; Brackets represent 95% confidence intervals.

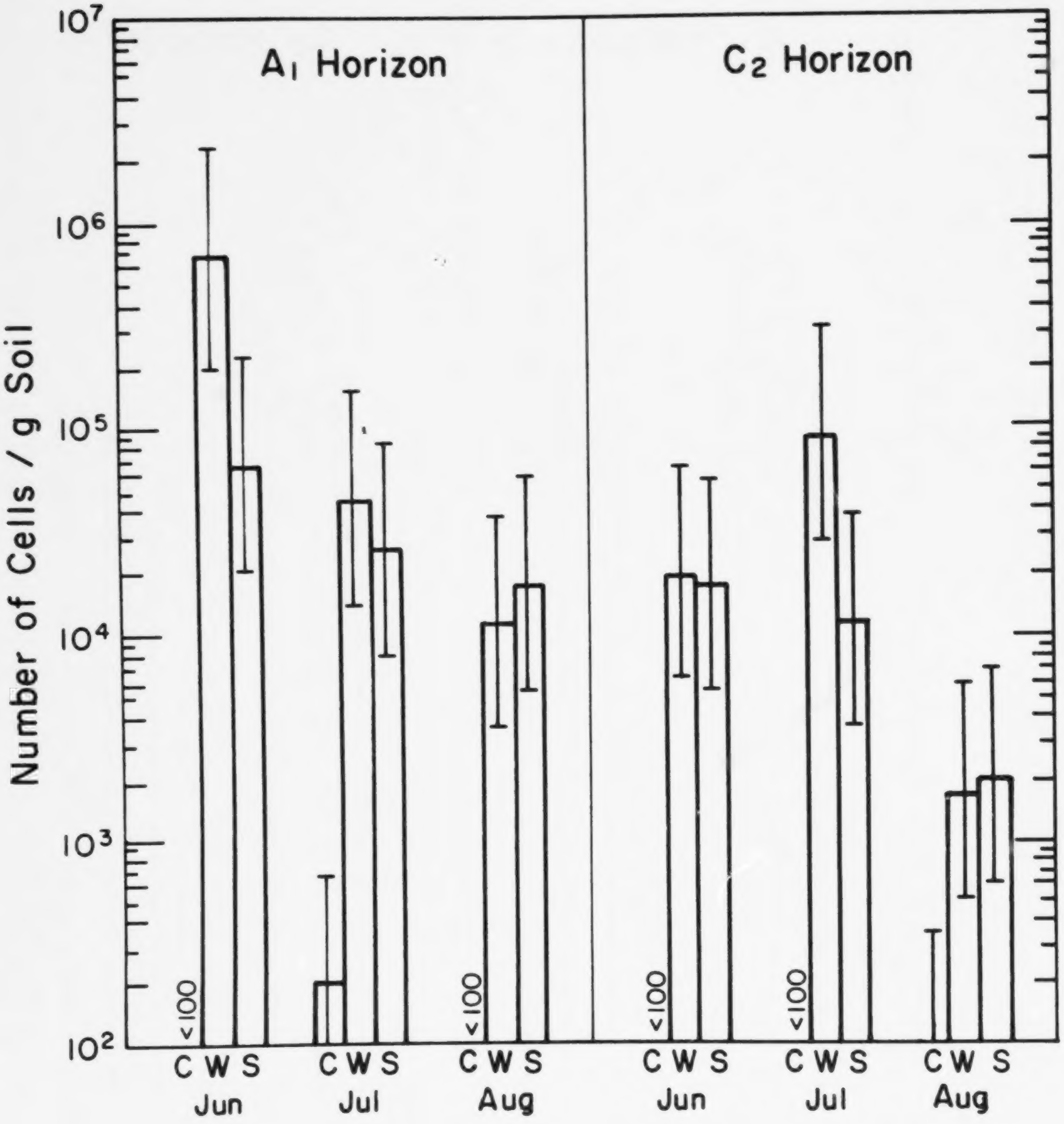


Figure 19. 1977 MPN counts of denitrifying bacteria in oiled and unoiled (control) plots. C = Control Plot; W = Winter Plot; S = Summer Plot; Brackets represent 95% confidence intervals.

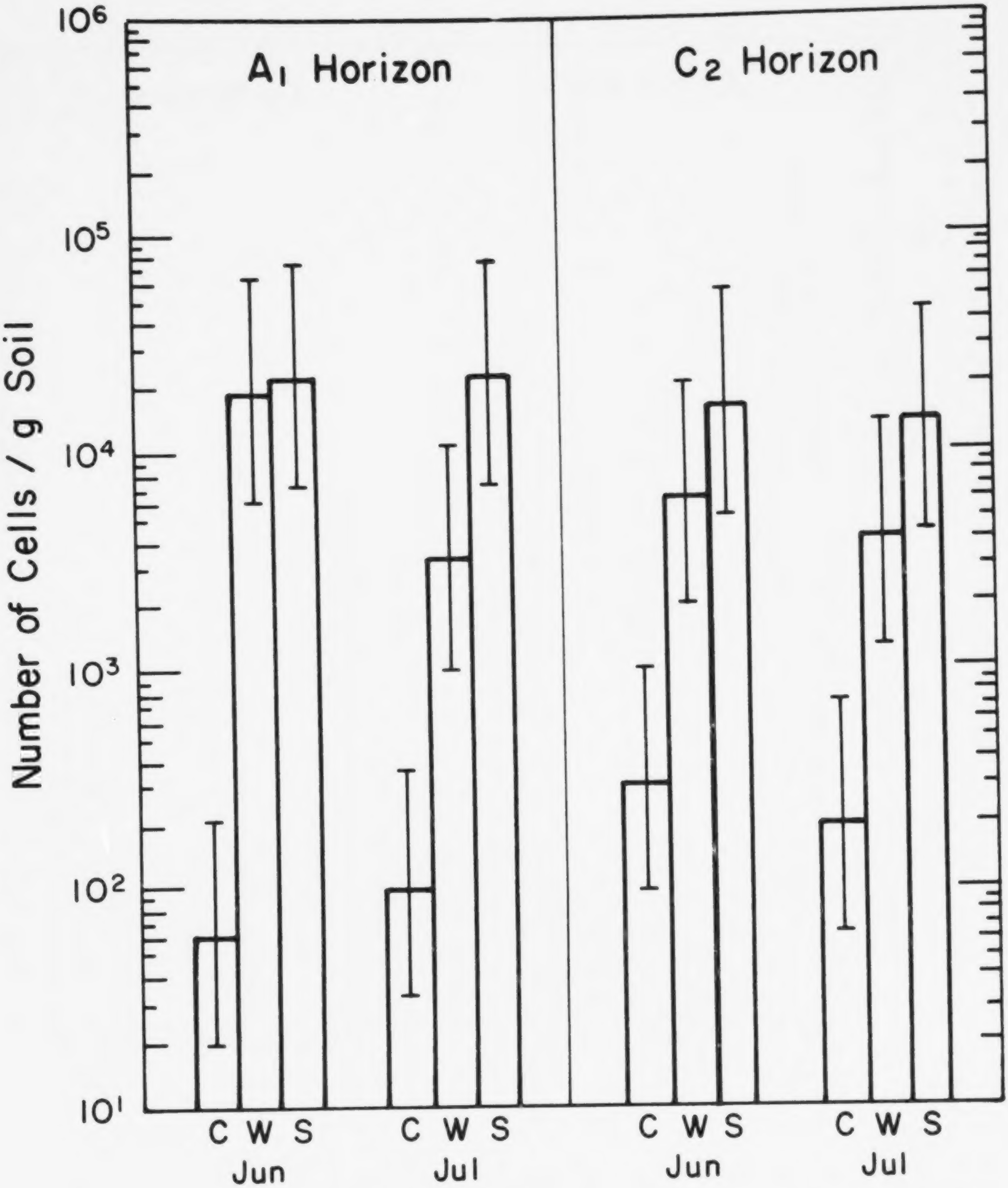


Figure 20. 1978 MPN counts of denitrifying bacteria in oiled and unoiled (control) plots. C = Control Plot; W = Winter Plot; S = Summer Plot; Brackets represent 95% confidence intervals.

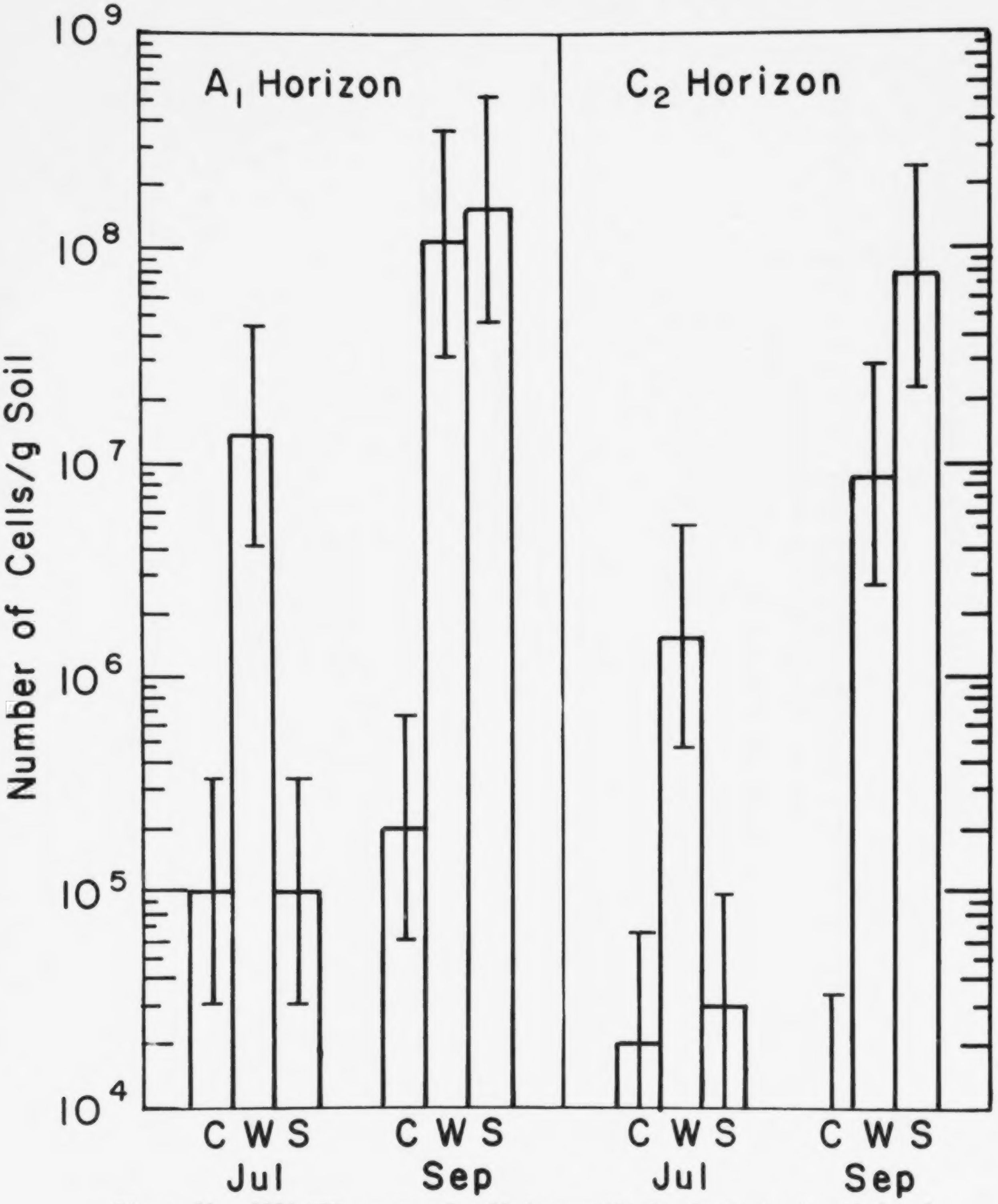


Figure 21. 1976 MPN counts of cellulose-utilizing bacteria in oiled and unoiled (control) plots. C = Control Plot; W = Winter Plot; S = Summer Plot; Brackets represent 95% confidence intervals.

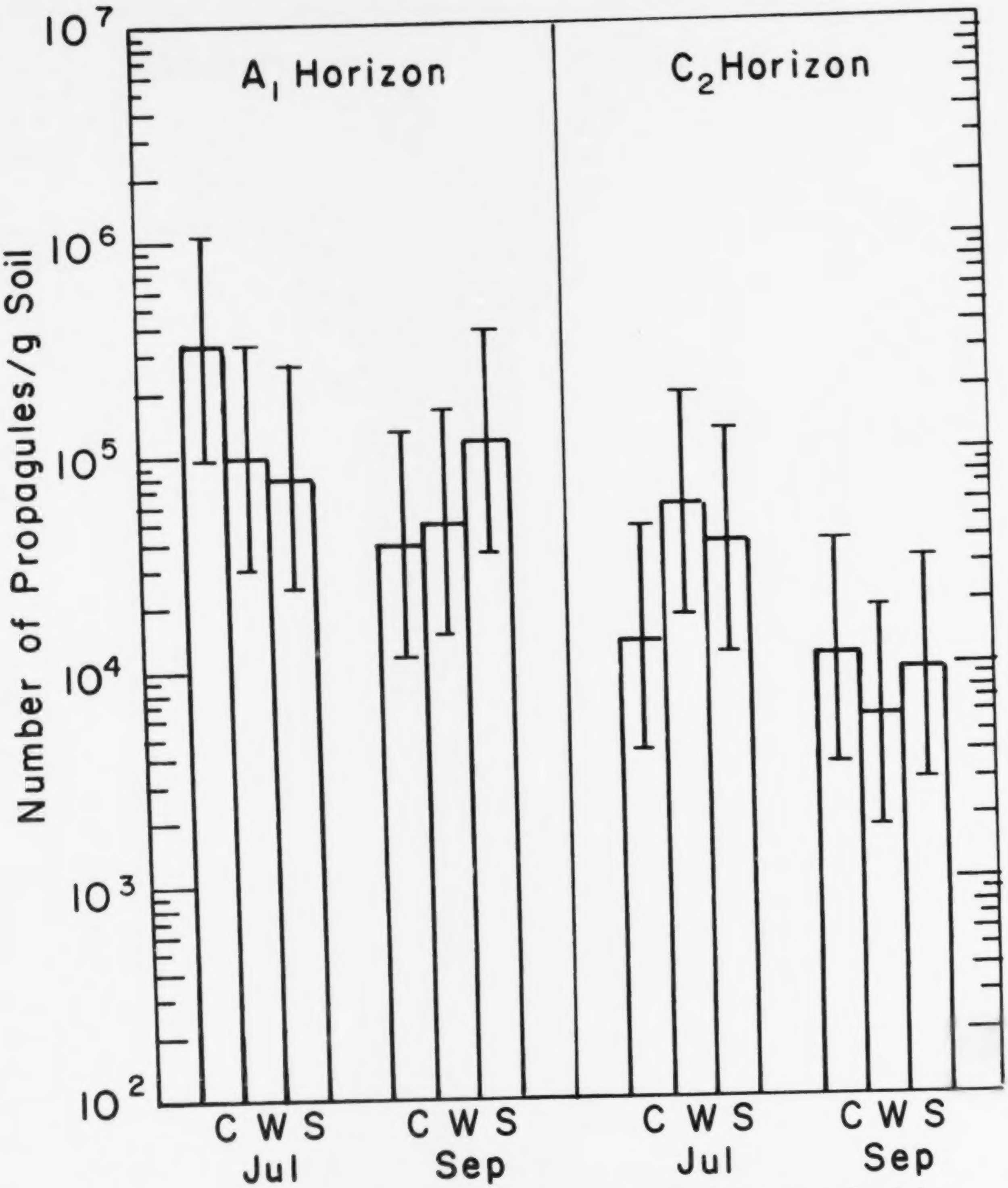


Figure 22. 1976 MPN counts of cellulose-utilizing filamentous fungi in oiled and unoiled (control) plots. C = Control Plot; W = Winter Plot; S = Summer Plot; Brackets represent 95% confidence intervals.

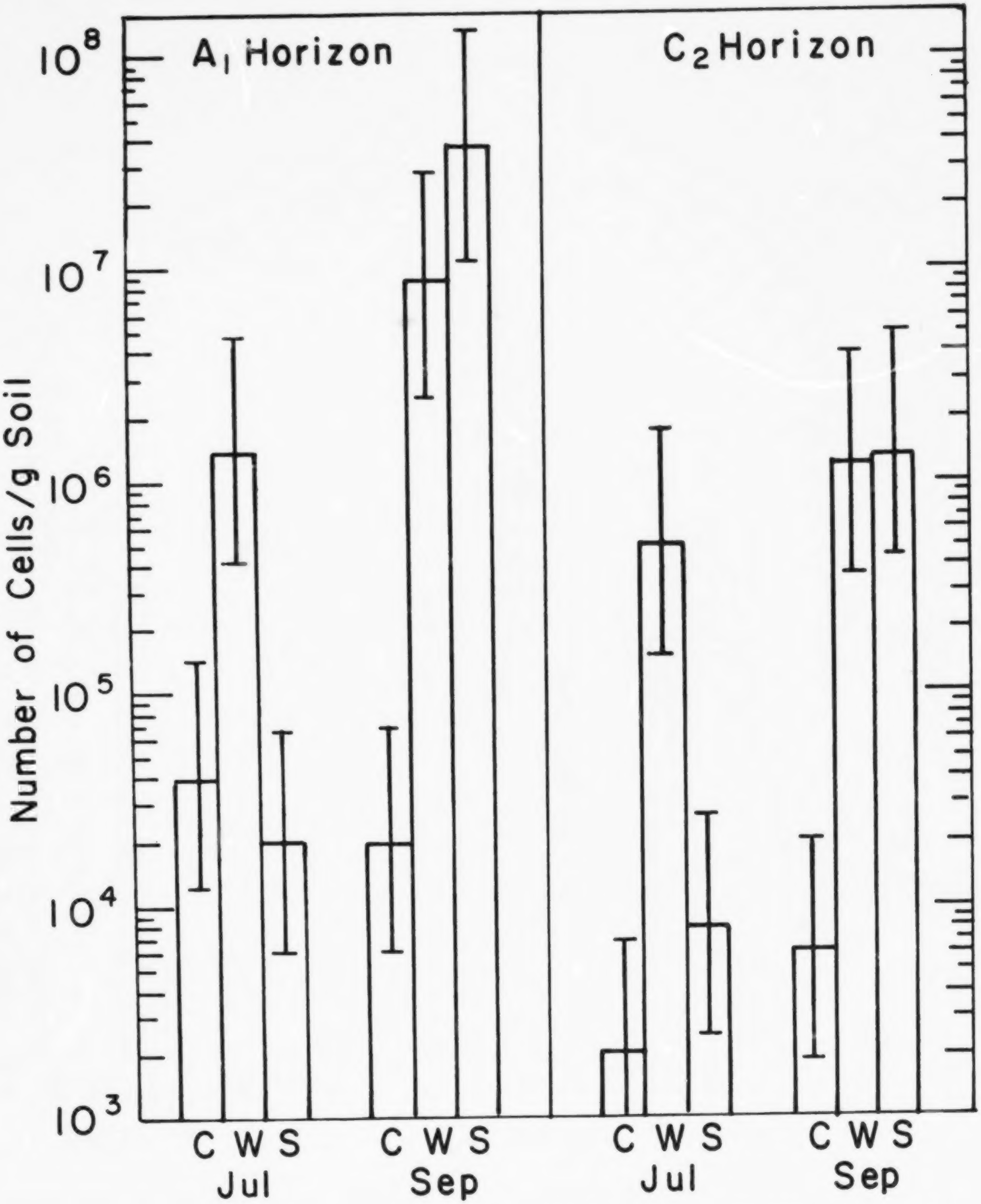


Figure 23. 1976 MPN counts of oil-utilizing bacteria in oiled and unoiled (control) plots. C = Control Plot; W = Winter Plot; S = Summer Plot; Brackets represent 95% confidence intervals.

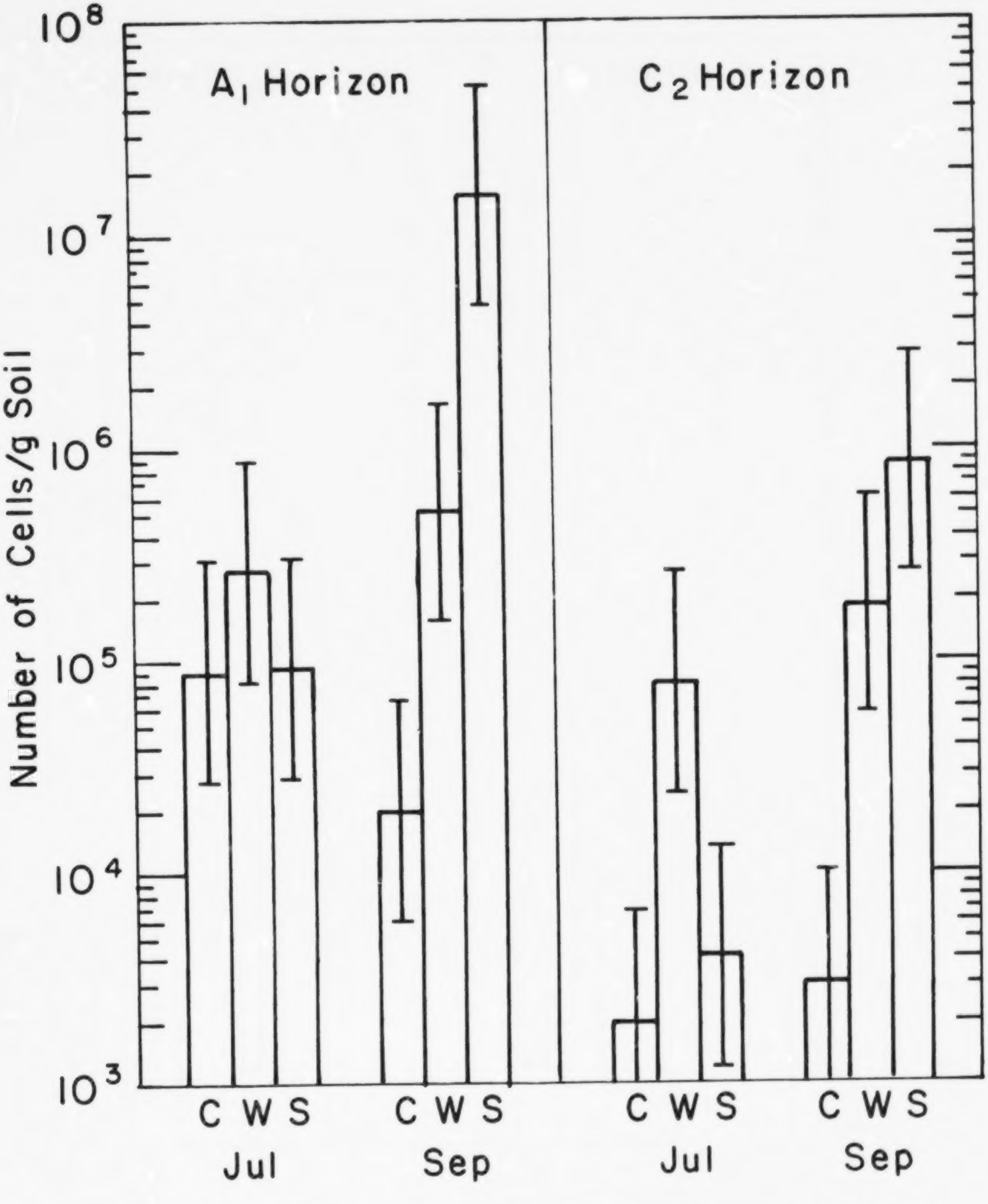


Figure 24. 1976 MPN counts of oil-utilizing yeasts in oiled and unoiled (control) plots. C = Control Plot; W = Winter Plot; S = Summer Plot; Brackets represent 95% confidence intervals.

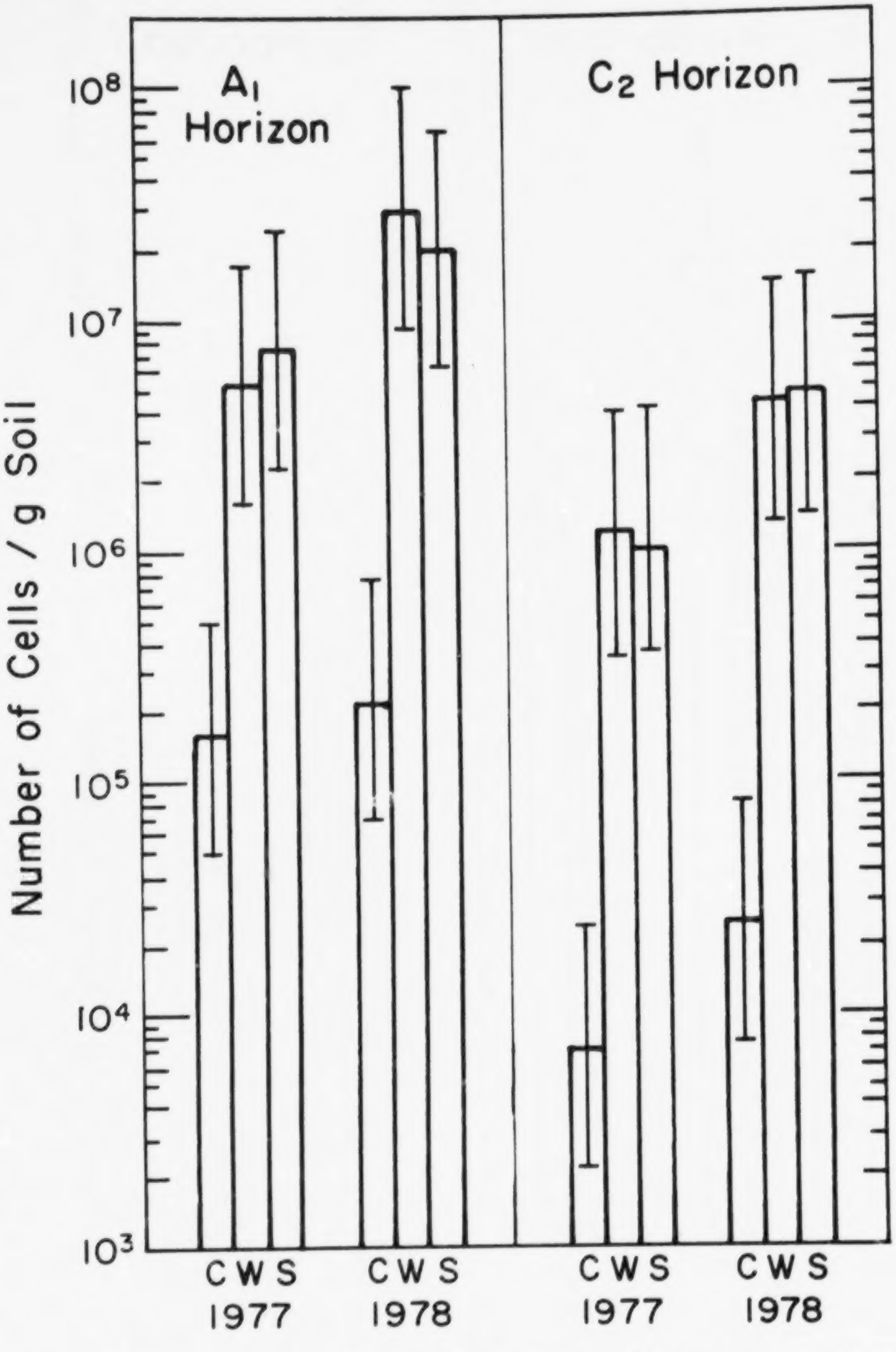


Figure 25. 1977 and 1978 MPN counts of oil-utilizing bacteria in oiled and unoiled (control) plots. C = Control Plot; W = Winter Plot; S = Summer Plot; Brackets represent 95% confidence intervals.

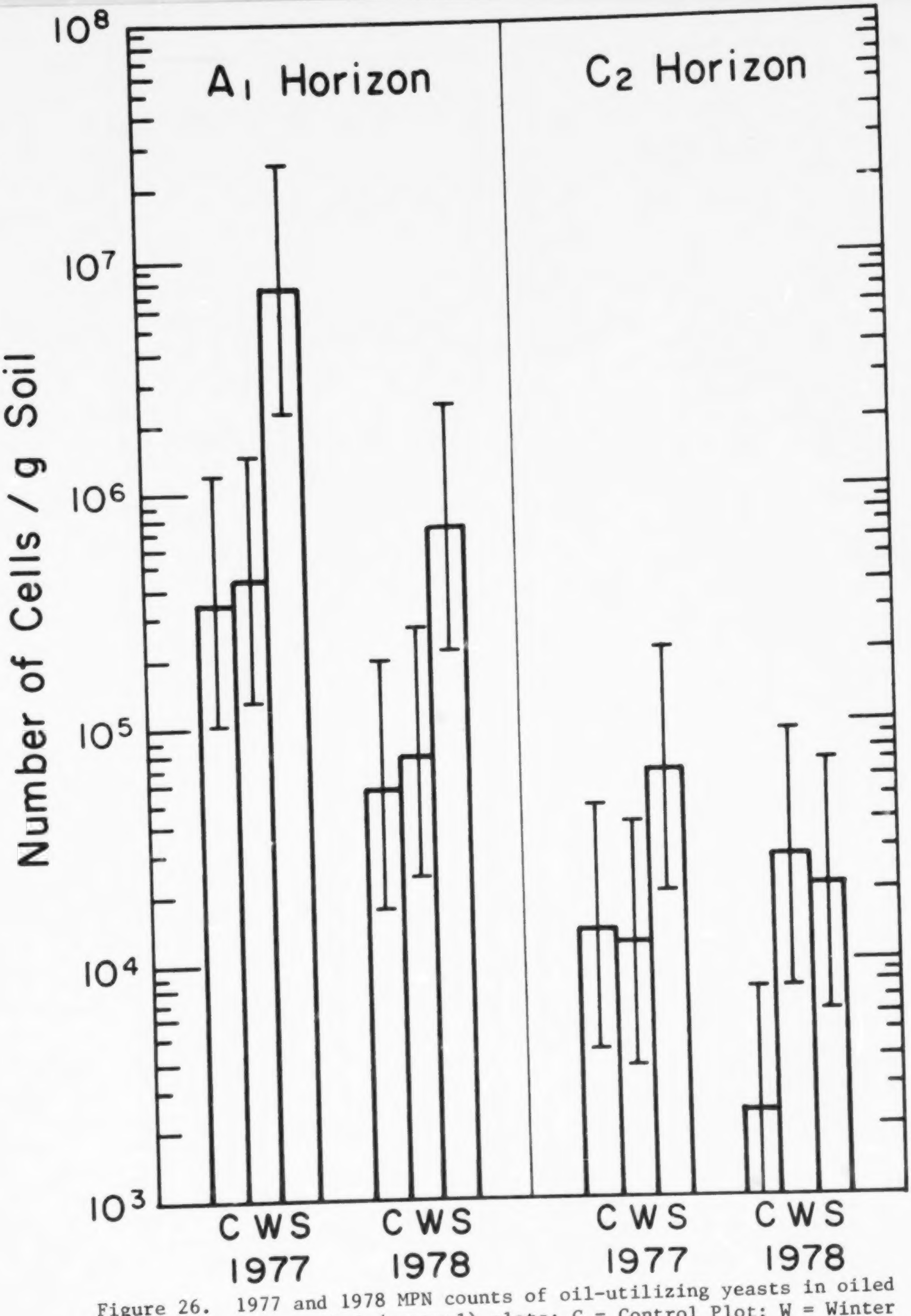


Figure 26. 1977 and 1978 MPN counts of oil-utilizing yeasts in oiled and unoiled (control) plots; C = Control Plot; W = Winter Plot; S = Summer Plot; Brackets represent 95% confidence intervals.

In 1976, counts of the oil-utilizing fungal populations in both oiled plots showed no significant change relative to the control (Figure 27). This trend continued through 1977 (Figure 28). In 1978 increases relative to the control plot occurred in both horizons in the summer plot and in the C_2 horizon of the winter plot. However, the increases may not be significant because of the unexplained decrease in control plot counts in 1978 relative to the preceeding years.

The effect of oil on *in vitro* soil respiration rate during 1976 is shown in Table 18. An immediate depression of soil respiration rates was apparent one day after the spill in both horizons of the winter plot and in the C_2 horizon of the summer plot. However, by the end of the growing season, the overall effect of oil on soil respiration rates was one of enhancement. The higher respiration rate in the A_1 horizon of the control plot in February relative to the other months is not too surprising in view of the evidence presented by Greenwood (1968) that freezing and thawing of soil results in increased organic matter decomposition.

The enhancing effect of oil on soil respiration observed in 1976 continued into 1977 (Table 19). This effect is greater in the A_1 horizon of the summer plot than in the winter plot, paralleling the enhancing effect on microbial populations.

The effect of oil spills on soil respiration *in situ* is shown in Table 19 and Figure 29. In 1976 and 1977, soil respiration rates were not significantly affected by the addition of oil in cylinders with the killed moss layer removed. However, in cylinders with the killed moss layer intact, respiration rates were significantly increased. These results suggest that the enhancing effect of oil on soil respiration *in situ* may be due to an increase in available substrate from plants killed as a result of the oil spill. It is interesting to note that the *in situ* respiration rates were higher in the summer plot relative to the winter plot in 1976 while the reverse was true in 1977. In 1976 (Table 20), respiration rates within a set of cylinders in the test plots had standard errors ranging from 0.31 to 1.81. This high variation may be due to a methodology artifact or more likely due to the heterogeneity of the soil within each plot. Because of the suspected heterogeneity in the test plots, 1977 *in situ* respiration measurements were pooled (Figure 29).

The soil pH of the site is generally 4.8 to 5.6. The addition of oil did not appear to cause a significant change in soil pH (Appendix, Table C-7. The soil water content in control plot and in both spill plots 5 m downslope from the point of oil application in 1976 through 1978 is presented in Tables 21 and 22. These measurements show distinct differences in water content among the three test plots. During 1977 and 1978 water content in both spill plots was less than the control except for one sample date. Furthermore, the water content in the winter plot was consistently lower relative to both the control and the summer plots.

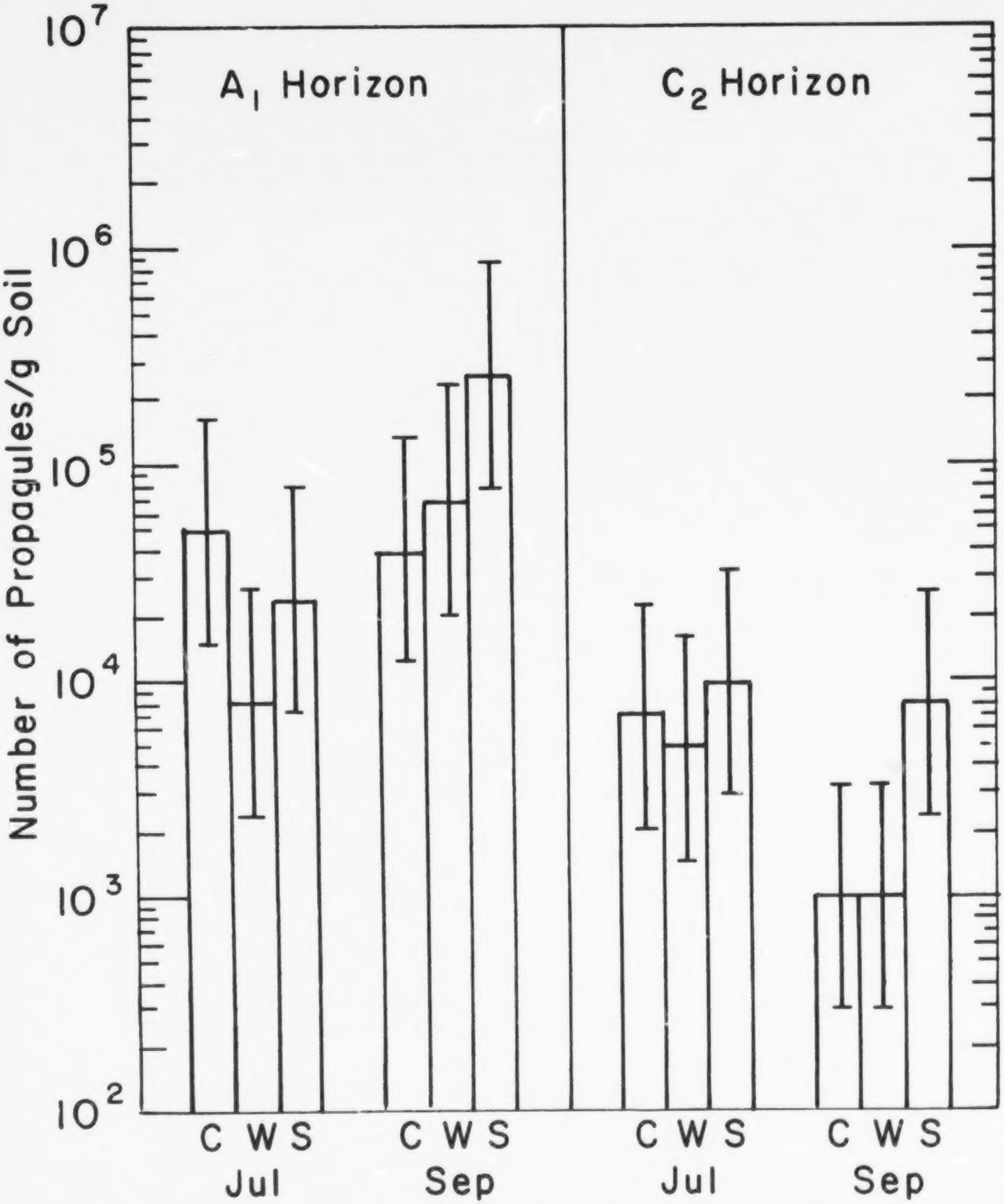


Figure 27. 1976 MPN counts of oil-utilizing filamentous fungi in oiled and unoiled (control) plots. C = Control Plot; W = Winter Plot; S = Summer Plot; Brackets represent 95% confidence intervals.

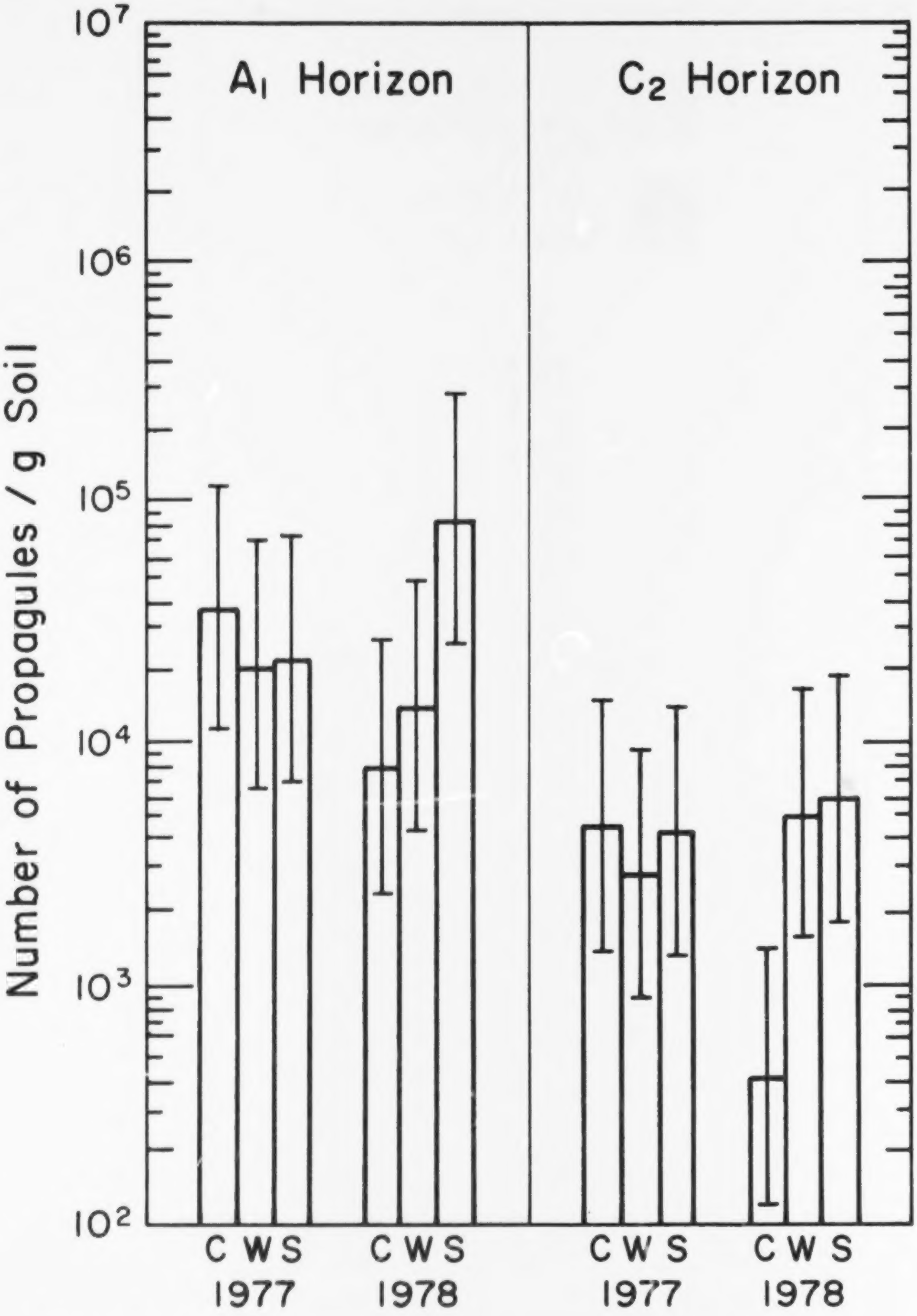


Figure 28. 1977 and 1978 MPN counts of oil-utilizing filamentous fungi in oiled and unoiled (control) plots. C = Control Plot; W = Winter Plot; S = Summer Plot; Brackets represent 95% confidence intervals.

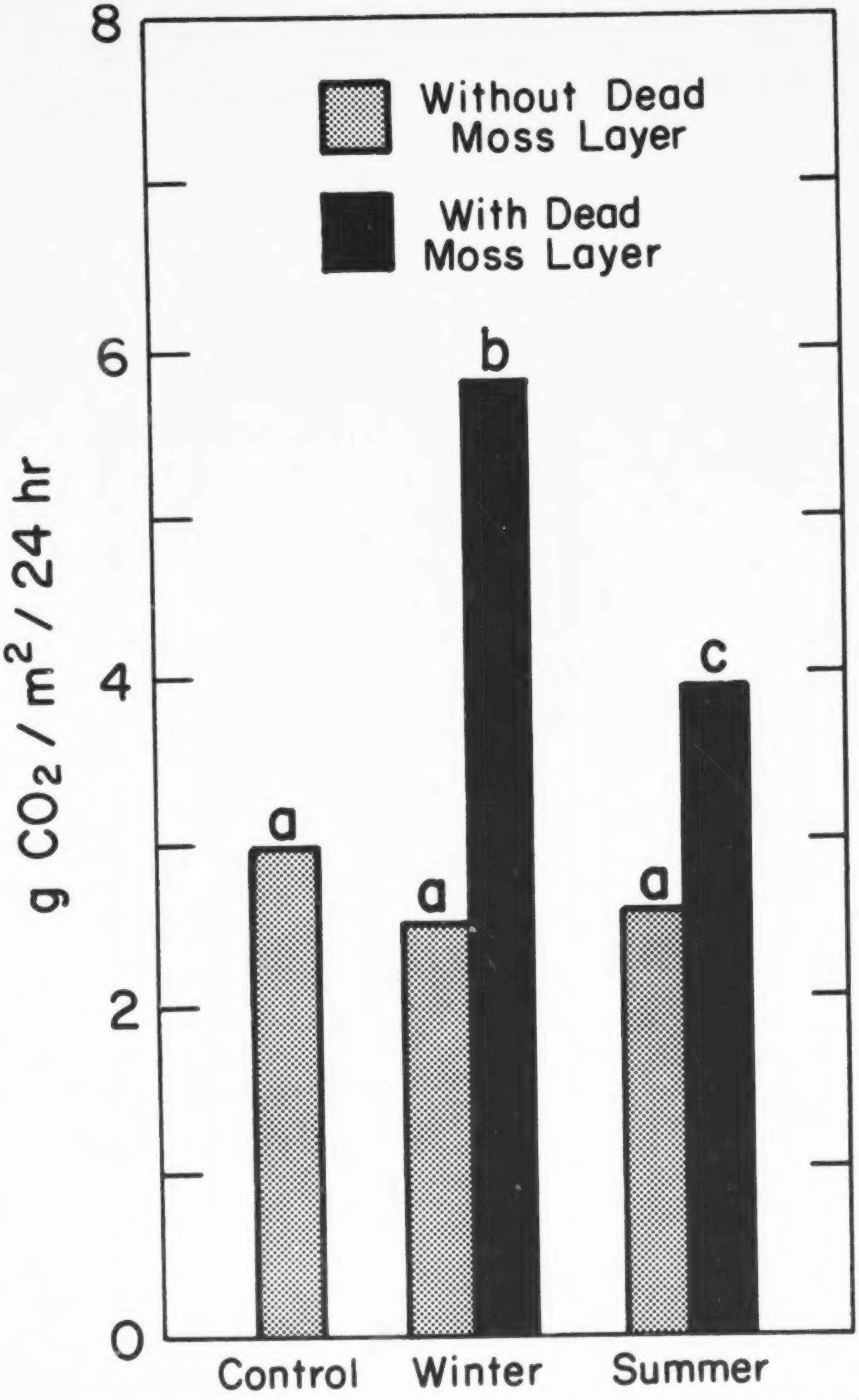


Figure 29. Respiration in situ in oiled and unoiled (control) plots in 1977. Histograms with dissimilar letters are significantly different at 1% level. (Respiration rates are means of three consecutive weekly determinations within each set of cylinders.)

TABLE 18. 1976 IN VITRO SOIL RESPIRATION RATES (mg CO_2/24 hr/100 g soil) IN OILED AND UNOILED (CONTROL) PLOTS.

Sampling time	Control plot	Winter plot	Summer plot
		A_1 Horizon	
February[a]	27.2	11.2**	-
July[b]	8.0	16.6**	20.2**
August	8.6	15.6**	30.6**cc
September	3.8	15.6**	37.8**cc
		C_2 Horizon	
February[a]	2.6	n.d.	-
July[b]	3.6	6.0**	1.2**cc
August	3.8	7.8**	6.2**
September	2.4	5.6**	13.6**cc

a Within 24 hours after winter oil spill.
b Within 24 hours after summer oil spill.
n.d. Not detectable by method used.
* Significantly different from the control at the 5% level.
** Significantly different from the control at the 1% level.
c Significantly different from the winter at the 5% level.
cc Significantly different from the winter at the 1% level.

TABLE 19. 1977 and 1978 IN VITRO SOIL RESPIRATION RATES (mg CO_2/24 hr/100 g soil) IN OILED AND UNOILED (CONTROL) PLOTS.

Sampling time	Control plot	Winter plot	Summer plot
1977		A_1 Horizon	
June	21.5	32.3**	79.9**cc
July	14.4	17.1*	35.1**cc
August	14.6	16.7	41.5**cc
		C_2 Horizon	
June	5.7	12.2**	25.6**cc
July	4.7	9.5**	8.5**
August	4.6	7.6**	6.6*
1978		A_1 Horizon	
July	19.7	24.1*	31.4**cc
July	6.6	10.3*	11.4**

* Significantly different from the control at 5% level.
** Significantly different from the control at 1% level.
cc Significantly different from the winter at 1% level.

TABLE 20. 1976 *IN SITU* SOIL RESPIRATION (g $CO_2/24\ hr/m^2$) IN OILED AND UNOILED (CONTROL) PLOTS.

Date	Control plot	Winter Plot		Summer plot	
	a	b	c	b	c
June 10	4.7	–	7.0	–	–
July 16	3.7	–	5.1	–	5.7
August 3	4.8	4.6	5.8	5.7	8.6*
August 12	2.2	3.3	4.8*	2.7	5.7**
September 1	2.9	1.6	4.0	4.5	6.4

a Respiration cylinders embedded after removal of live moss layer.
b Respiration cylinders embedded after removal of dead moss layer.
c Respiration cylinders embedded without removal of dead moss layer.
* Significantly different from control at 5% level.
** Significantly different from control at 1% level.

TABLE 21. 1976 SOIL WATER CONTENT (%) IN OILED AND UNOILED (CONTROL) PLOTS.

Sampling time	Control plot	Winter plot	Summer plot
		A_1 Horizon	
February[a]	295	154*	–
June	214	119*	–
July[b]	155	122**	171* cc
August	113	96	162**cc
September	95	81**	153**cc
		C_2 Horizon	
February[a]	56	70	--
June	114	56**	–
July[b]	71	71	99**cc
August	62	63	53**cc
September	55	50	83**cc

a Within 24 hours after winter oil spill.
b Within 24 hours after summer oil spill.
* Significantly different from control at 5% level.
** Significantly different from control at 1% level.
cc Significantly different from winter at 1% level.

TABLE 22. 1977 and 1978 SOIL WATER CONTENT (%) IN OILED AND UNOILED (CONTROL) PLOTS.

Sampling time	Control plot	Winter plot	Summer plot
1977		A_1 Horizon	
June	243	102 **	177 ** cc
July	168	77 **	137 * cc
August	194	74 **	137 ** cc
		C_2 Horizon	
June	92	62 **	105 ** cc
July	62	48 **	58 * cc
August	60	46 **	55 ** cc
1978		A_1 Horizon	
June	159	97 **	190 ** cc
July	146	86 **	113 ** cc
		C_2 Horizon	
June	64	59 **	70 ** cc
July	58	55	51 **

* Significantly different from the control at 5% level.
** Significantly different from the control at 1% level.
cc Significantly different from the winter at 1% level.

The concentrations of benzene-extractable oil in the oiled soil samples are presented in Table 23. It is apparent that the oil concentrations in the A_1 horizon were higher than those in the C_2. The vertical migration of oil through the soil horizon with time is evident, the distribution of oil in the soil layers of the winter plot being different from the summer.

Although the concentration of oil was greater in the A_1 layer, the impact of oil was not limited to this horizon. Both A_1 and C_2 layers showed significant increases in microbial populations and respiratory activity, and in the case of the filamentous fungal population, significant reductions immediately following and up to the third growing

TABLE 23. OIL CONTENT (%) IN SOIL 5 M DOWNSLOPE FROM POINT OF OIL APPLICATION

Sampling time	Winter plot	Summer plot
1976	A_1 Horizon	
February[a]	8.0	-
June	17.9	-
July[b]	8.8	12.2
August	4.3	6.3
September	5.1	6.5
	C_2 Horizon	
February[a]	0.2	-
June	0.1	-
July[b]	1.5	1.3
August	1.0	0.2
September	1.4	0.9
1977	A_1 Horizon	
June	12.5	25.0
July	4.7	9.2
August	4.5	6.8
	C_2 Horizon	
June	2.2	3.3
July	2.9	0.8
August	1.6	0.8
1978	A_1 Horizon	
June	9.3	10.7
July	9.0	8.0
	C_2 Horizon	
June	2.1	0.9
July	1.4	0.3

[a] Within 24 hours after winter oil spill.
[b] Within 24 hours after summer oil spill.

season after the oil spills. However, the response in the C_2 layer was sometimes slower to appear. In comparing the enhancing effect of oil in the two horizons, its extent was generally greater in the A_1 than in the C_2 layer.

The relationship of respiration rates to microbial counts and the relationship of oil concentrations to microbial counts and respiration rates were also examined. Significant linear correlations were found between *in vitro* soil respiration rates and numbers of heterotrophic bacteria or filamentous fungi (Appendix, Tables C-8, C-9, C-10, and C-11). Similarly, in the oiled plots, significant linear correlations were found between concentrations of oil and *in vitro* soil respiration rates or microbial numbers (Appendix, Tables C-14 to C-21).

The data indicate that the soil microbial populations showed a rapid and differing response during the first year after the oil spills. Immediately after the summer spill the initial depression in numbers of heterotrophic bacteria and filamentous fungal propagules, and in respiratory activity appear to be due to toxicity of volatiles present in the crude oil. At the time of the spill in July, concentrations of volatiles in the summer plot exceeded those in the winter plot. However, loss of volatiles in the spilled oil was faster in the summer plot than in the winter plot. By the second growing season, greater concentrations of volatiles are present in the soil in the winter than in the summer plot.

At the end of the first growing season in the summer plot, counts of filamentous fungi and heterotrophic bacteria, and soil respiratory activity significantly exceeded initial summer and control levels. In the winter plot, numbers of filamentous fungal propagules were reduced immediately after the spill and remained depressed throughout the initial plant growing season, although bacterial counts and soil respiration rates were significantly higher than in the control by the end of the first season. During the second and third growing seasons, significant reductions in filamentous fungal propagules were evident not only in the winter plot but also in the summer plot while significant increases in counts of heterotrophic bacteria and yeasts occurred.

It is apparent that filamentous fungi are more sensitive to the presence of oil and in fact were adversely affected by the oil spills. The extent of inhibition by the oil was greater in the winter plot than in the summer plot. The effect of oil on this microbial population could be due to direct toxicity of the oil components or may be related to the aeration status in the oiled plots. Significant amounts of volatiles, particularly the C_5-C_8 fractions, were found up to the second growing season in the organic and mineral soil layers of both oiled plots. Concentrations were higher in the winter plot. Other workers (Teh and Lee, 1973, 1974) have shown that mycelial growth and spore germination of some fungi are inhibited by n-hexane, n-heptane and n-octane which are components of the volatile fraction of oil. Also, filamentous fungi as a group are strict aerobes (with a few exceptions). When oxygen diffusion becomes inadequate to meet the microbial demand for aerobic metabolism, the fungi are first to suffer (Alexander, 1977). The significant increases in numbers of anaerobic and denitrifying bacteria in the oiled plots suggest the possibility of decreased oxygen availability in the oiled plots. The differences in the mode of flow, concentration, and physical properties of the spilled oil in the winter and summer plots may account for differences in aeration status.

Because of oil damage on higher and lower plants shown by other studies (Hunt *et al*., 1973; McCown *et al*., 1973; Miller *et al*., 1978; Antibus and Linkins, 1978), the input of plant residues into the soil system in oil perturbed areas may be considerable. In this study, during the first year of the spills, damage to vegetation was similarly observed, the damage being more rapid and extensive in the summer plot (in the heavily oil impacted areas). This coincides with our finding of greater increases in microbial populations and soil respiration rates in the summer plot compared to the winter plot. This suggests that the increase in levels of soil microbial populations and activity may be due not only to the presence of petroleum substrate but also to the presence of substrates released from dying or dead plants. It can be postulated that readily available carbon substrates are released rapidly by the solvent action of oil on plant cell membranes. The burst of microbial growth and respiratory activity observed shortly after the oil spills may have been triggered by the sudden release of readily utilizable carbon substrates from plants in addition to those present in the oil. A lag time may occur before oil is utilized because of the time needed for development of microbial populations capable of degrading oil and because of the adaptive nature of hydrocarbon enzymes (Zobell, 1950; Van der Linden and Thijsse, 1965). However we did not determine this in our study. We did observe significant increases in oil-utilizing bacteria and yeasts two months after the summer spill and five months after the winter spill. Increases in these populations may have occurred sooner but earlier enumerations were only done 24 hours after the summer spill.

Soil respiration rates measured in the test plots give an index of organic matter decomposition under field conditions. The data on *in situ* respiration showed an enhancement of soil respiratory activity in the presence of oil and plants killed by the oil but no effect on respiration when killed plant material was removed. This suggests that the enhancing effect of oil on 1976 and 1977 soil respiration *in situ* is indirect, that increased respiration may be due to increased decomposition of carbon substrate released when vegetation was damaged by the oil spills.

This study has shown that growth of some microbial populations has not been adversly affected. For the most part, growth has been increased by the oil applications. This suggests that nutrient transformations carried out by these populations may be stimulated. However, the actual activity of these functional groups is determined by a balance of the physiological requirements of the microorganisms and the existing environmental conditions. It is interesting to note that in soil samples collected from the treatment plots, microbial counts correlated significantly with metabolic activity, i.e. soil respiratory activity.

Future studies are required to determine the effects of spilled oil on different microbial processes in the carbon and nitrogen cycles, such as nitrogen-fixation, denitrification, nitrification and ammonification, and cellulose degradation.

The soil microbial populations showed a differential response through the third growing season after the hot oil spills. Although the effects ranged from inhibition to stimulation, stimulation of growth of the indigenous soil microorganisms and their activities seems to predominate. Effects of oil appear to be both direct and indirect but mechanisms involved need further study. Additional information is necessary to determine whether microbial populations continue to sustain their levels as oil damaged plant substrates are depleted, and whether they may then utilize oil as the main substrate. With the vast amount of carbon present in crude oil, it is inevitable that nutrient limitations will occur. Consideration must be given to type and timing of nutrient additions and to other means of enhancing natural recovery processes such as improving aeration.

Oil Effects on Vegetation

Following application of the oil, vegetation damage was assessed visually via changes in leaf color and leaf fall. There were three main time frames for injuries: 1) immediate; 2) occurring during the initial growing season; and 3) cumulative, occurring after the initial growing season. Toxicity probably caused immediate injuries while delayed injuries may either be due to indirect (physical) effects or to a combination of toxic and indirect effects.

Virtually all above ground foliage that came into contact with the oil was quickly killed. Turbidity was immediately reduced and foliage appeared dead within several days. In the winter spill the foliage was dead when it was first observed in mid-May after snowmelt. Evergreens such as cranberry retained the damaged foliage longer than deciduous species such as birch. The zone of contact was generally limited to the immediate areas below the 5-m wide oil feeder and to areas of low relief in the path of aboveground flowing oil (Figure 9b). Lichens and mosses, which tend to be concentrated in low areas and have a low-growth form, suffered particularly heavy mortality. In contrast, cottongrass tussocks with a raised, upright growth form and species growing on areas of higher relief kept most of their aboveground biomass above the oil. These species continued to grow and flower, at least initially, despite their being surrounded by oil (Figure 30).

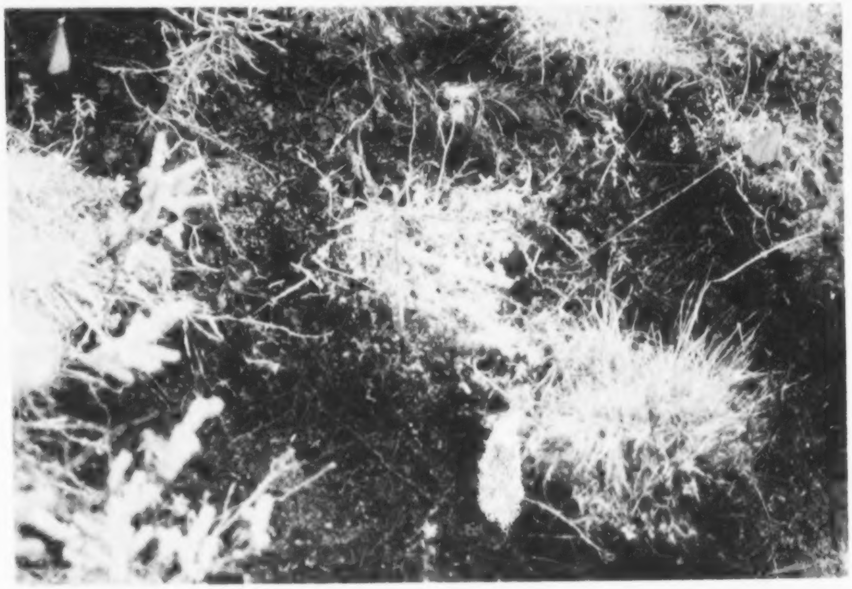


Figure 30. Cottongrass tussocks growing despite being surrounded by surface oil and dead moss and lichens.

Since oil flowed into depressions and low areas, aboveground vegetation on higher relief was not in physical contact with the oil. Belowground contact was difficult to accurately ascertain since the nature of the study would not permit large-scale destructive sampling of roots and soil.

However, the soil pits dug in August, 1978 provided some evidence of species differences in rooting habits and the extent of belowground oil contact (Figure 31). Very few (less than 10%) roots extended into the mineral soil (C_2) and these were all observed to be less than 1 mm in diameter. Some species (Vaccinium uligonosum, Rubus chamaemorus, Picea mariana) had roots which were concentrated in the 0_1 and upper 0_2 soil horizon but extended into both the A_1 and C_2 horizons. Since the oil in most cases flowed within the 0_2 horizon it contacted roots of most species to at least some extent.

In contrast to the other species observed on the site, cottongrass tussock roots have primarily a vertical orientation instead of the more commonly observed horizontal growth. Eriophorum vaginatum produces new

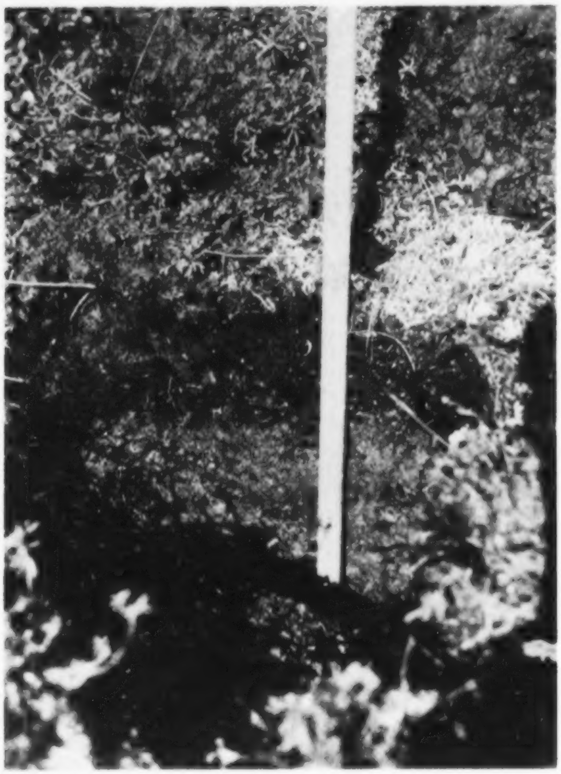


Figure 31. Soil pit for determining rooting habits and belowground oil movement. (Note dark band of oil just above light colored mineral soil.)

roots annually which grow down following the receding frozen soil layers as the growing season progresses (Chapin et al., 1979). In a soil pit 34 m downslope on the summer spill plot, cottongrass roots that appeared vigorous and white had grown into the C_2 horizon after penetrating 9 cm of oil soaked soil, mainly within the O_2 horizon (Figure 32). Thus cottongrass, by both its aboveground tussock growth form and its below ground vertical rooting strategy minimizes the amount of biomass which is in contact with oil. This undoubtedly helps to account for the low mortality of cottongrass in the contaminated areas.

Damage during the first growing season varied with such factors as species, position on slope, growth form, and season of spill. In general, delayed injuries first appeared on foliage that was above the path

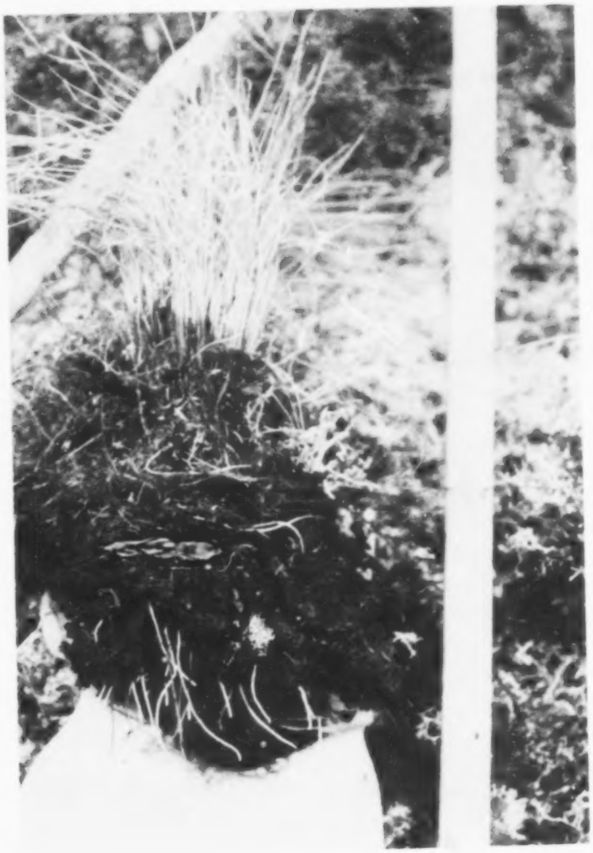


Figure 32. Cottongrass (Eriophorum vaginatum) roots growing through oil soaked soil.

of the oil and near the top of the plot. Injuries subsequently appeared farther downslope and in areas of subsurface flow immediately adjacent to surface oil. Deciduous leaves turned brown and abscised prior to evergreen leaves. For example, in the summer spill, resin birch foliage turned brown 5 m downslope two weeks after the spill, but did not turn brown at 10 m until four weeks after the spill. In contrast, Labrador tea and cranberry leaves had only turned partially brown 10 m downslope at the end of the growing season (six weeks). Black spruce had some of the most delayed damage of any species. Although spruce in the upper 2 m of the winter spill began dropping needles by mid-June, the needles had not entirely dropped until September. On the summer spill the needles turned brown on several spruce and many had chlorotic foliage by September. Most needles remained on the trees at that time.

The nutrient analysis of black spruce foliage indicated a significant decrease from 1975 to 1976 in the total nitrogen content of the new foliage of trees exposed to oil (Table 24). Total phosphorus and total potassium did not show consistent changes. Although there was significant year-to-year variability in total nitrogen, the magnitude of the nitrogen decrease on the oiled plots was great enough to be statistically significant at the 5% level when compared with either the control plot during the same year or with the same plot from the previous year when it was unoiled. The decrease in nitrogen content may be due to: 1) disruption of the metabolism of root nutrient uptake; 2) coating of the roots by a hydrophobic layer of oil which physically interferred with nitrogen uptake; 3) immobilization of nitrogen by increased microbial growth, in response to addition of readily utilizable carbon substrates, or 4) some combination of these effects.

TABLE 24. NUTRIENT CONTENT OF BLACK SPRUCE FOLIAGE.

Year	Treatment	Avg/Std.Error %N*	Avg/Std.Error %P*	Avg/Std.Error %K*
1975	Control	0.91 (.03) a	0.07 (.01) a	0.56 (.04) a
(unoiled)	Summer	0.90 (.02) a	0.08 (.01) a	0.54 (.01) a
	Winter	0.94 (.03) a	0.09 (.00) a	0.57 (.01) a
1976	Control	1.05 (.04) a	0.11 (.00) a	0.35 (.00) a
(oiled)	Summer	0.75 (.06) b	0.10 (.01) a	0.32 (.01) b
	Winter	0.86 (.02) b	0.10 (.00) a	0.37 (.00) a

* Figures with a common letter are not significantly different at the 5% level using the t-test. Comparisons are made within years and within columns.

Cumulative injuries were largely limited to evergreen species. During the second growing season increasing amounts of black spruce foliage became chlorotic, turned brown, and abscised. Similarly, foliage of cranberry and Labrador tea continued to turn red and then brown throughout the second growing season so that some injuries were apparent as far downslope as 25 m on the winter spill and 35 m on the summer spill. Cumulative injuries could be due to a combination of direct oil-caused stress and problems of overwintering. This mechanism has also been suggested by other studies (McCown _et al._, 1973; Linkins and Antibus, 1978).

The number and distribution of the killed black spruce trees and saplings due to the oil is shown in Table 25. The total number of black spruce trees killed over three growing seasons is similar on both spills, but mortality was more delayed on the winter spill.

TABLE 25. MORTALITY OF BLACK SPRUCE

	Thru 1977		1978		
	Max. Distance Downslope (m)	Total Trees Killed	Max. Distance Downslope	Total Trees Killed	3 Year Total Mortality
Summer Spill	10	26	10	2	28
Winter Spill	9	20	20	10	30

The roots of the black spruce are concentrated in the O_1 and O_2 horizons and extend further away from the stem than do the roots of most smaller species. Therefore the black spruce are not as likely as other species to have their entire root systems surrounded by oil but are more likely to have some roots in contact with the oil. The very slow mortality of the black spruce may be due in part to chronic toxicity or physical stresses on a portion of the root systems that continues to increase for three or more years (Freedman and Hutchinson, 1976; Hutchinson and Freedman, 1978).

Table 26 presents a comparison of vegetation data from the permanent quadrats before and after the winter and summer spills. Quadrats were grouped into 3 categories: 1) areas impacted by surface flows of oil, 2) areas impacted by subsurface oil flows, and 3) controls - undisturbed areas. Surface flow areas showed the most severe effects in both spill plots. Live ground cover was most reduced on the upper 5 m of the winter spill but was also reduced on most of the surface oiled summer spill.

It is difficult to accurately compare the summer and winter spill quadrats because the oil flowed off to the side of the winter plot. Only three permanant quadrats on the winter spill were impacted in comparison to twelve on the summer spill. However, damage was more severe in both spill plots on areas of surface flow where the oil could contact both above and belowground plant parts. There were 76.3 m^2 of surface flow on the winter spill in comparison to 30.3 m^2 on the summer spill. This would imply that the real extent of severe vegetation injury (as defined by a decrease in total live ground cover) was greater on the winter spill. Stem counts of shrub species readily indicated

TABLE 26. PRE (1975 & 1976) AND POST-SPILL (1978) VEGETATION ANALYSIS (AVE. CHANGE)

	Control	Winter Spill		Summer Spill	
Species or Category		Surface flow	Subsurface flow[a]	Surface flow	Subsurface flow
Oiled ground-cover*	0	+80	0	+43	0
Frequency of occurrence	0,0	0,100	0,0	0,100	0,0
Trees					
Picea mariana (trees)-cover	0	-50	-10	-10	-5
number of individuals	0	-2	-	-15	-1
occurrence	43,43	100,0	0,0	33, 17	33, 17
(saplings) cover	-33	-40	-	-15	0
number of individual	0	-4	-	-1	-.5
occurrence	43,43	100,0	0,0	33, 17	33, 33
Medium and low shrubs					
Betula glandulosa-cover	0	-20	0	-15	+23
number of stems	-.6	-4	0	-7.5	-3.7
occurrence	100, 100	50, 0	100, 100	100, 67	100, 200
Ledum groenlandicum-cover	-4	-25	+10	-5.6	+2
number of stems	-24	-82	-5	-25.7	-6.4
occurrence	71, 71	100,0	100, 100	100, 83	83, 83
Ledum decumbens-cover	0	-	-10	-4	+1.7
number of stems	-14	-	-4	-17.6	-17.2
occurrence	86, 86	0,0	100, 0	83, 67	100, 100
Vaccinium uligonosum-cover	-2.9	-30	-20	-5.6	0
number of stems	-5.3	-35.5	-105	-39.3	-13.7
occurrence	100, 100	100, 0	100, 100	100, 83	100, 100
Vaccinium vitis-idaea L.-cover	-43	-25	0	-10	+3.3
occurrence	100, 100	100, 50	100, 100	100, 100	100, 100
Dwarf shrubs and herbs					
Rubus chamaemorus L.-cover	0	-10	0	-8.3	+5
number of leaves	+.25	-3.5	-5	-15.8	-6.2
occurrence	57, 57	100, 50	100, 100	100, 33	100, 83
Equisetum sylvaticum L.-cover	-10	-10	-10	-10	-10
occurrence	71, 57	100, 0	100, 0	50, 0	33, 0

TABLE 26. (Cont'd)

Species or Category	Control	Winter Spill		Summer Spill	
		Surface flow	Subsurface flow[a]	Surface flow	Subsurface flow
Petasites hyperboreus	+3.3	-10	-20	-	-
Rydb.-cover					
number of leaves	+.67	-.15	-3	-	-
occurrence	14, 28	100, 0	100, 0	0,0	0,0
Eriophorum vaginatum	-5.7	-15	0	0	0
-cover					
occurrence	100, 100	100, 50	100, 100	100, 83	100, 100
Graminae: cover	+14	-	-	+1.4	-
occurrence	0, 14	0,0	0,0	0, 14	0,0
Mosses					
Total: cover	-1.7	-60	+10	-8.3	+10
occurrence	100, 100	100, 50	100, 100	100, 100	100, 100
Pleurozium schreberi	-8.6	-35	+10	-10	+5
(Brid) Matt-cover					
occurrence	100, 100	100, 50	100, 100	100, 100	100, 100
Polytrichum spp-cover	-1.4	-20	-10	-4	-3.3
occurrence	100, 71	100, 0	100, 0	83, 60	50, 100
Dicranum spp-cover	-4.3	-20	+10	-5	+1.7
occurrence	86.71	100, 0	100, 100	67, 50	100, 100
Sphagnum spp-cover	+5	-	-	-6.7	+10
occurrence	57, 57	0,0	0,0	100, 100	83, 83
Lichen					
Total: Cover	-12.9	-35,	0	+1.7	+6.7
occurrence	100,	100, 50	100, 100	100, 100	100, 100
Cladonia spp-cover	-8.7	-30	0	+1.7	+5
occurrence	100,	100, 50	100, 100	100, 100	100, 100
Cetraria spp-cover	+1.4	-20	+10	-5	-4
occurrence	100,	100, 50	100, 100	100, 67	83, 50
Peltigera spp-cover	-10	-20	-10	+6	-2
occurrence	100,	100, 0	100, 0	50, 100	83, 83
Total live ground cover	0	-60	0	-16.7	-3.3

* Ave. change in % vegetation cover
Pre-spill followed by post-spill frequency
a:1 quadrat only

injury within surface flows. Blueberry was especially susceptible (Table 26). This could be explained by its very shallow rooting habit. Resin birch and both species of Labrador tea were also readily injured. Mosses and lichens were completely killed in quadrats with 80% of oiled ground cover, but were only reduced in areas of less extensive surface flow. Damage was most severe in the upper 10 m of the spills. Within this area surface flows generally covered a greater percentage of the ground and the penetration of the oil into the soil was greater (Figures 10 a-e).

Areas of subsurface flow had much less damage. Live ground cover was not reduced. Mosses and lichens appeared healthy but shrubs and black spruce were sometimes affected. Blueberry and cloudberry were reduced in number of live stems while some black spruce trees and shrubs were killed.

In summary, damage appeared more rapidly on the summer spill. This could have been due to the more rapid flow downslope or the oil remaining hotter for a longer period. Both of these factors could increase the rate of uptake of toxic components by plant roots. In addition, actively growing vegetation was subjected to greater contact with volatiles on the summer spill since the vegetation was dormant at the time of the winter spill. Many of the volatile components were lost prior to the start of the first growing season following the spill on the winter plot (Figure 13 and Table 3). On both plots deciduous species showed a more rapid browning of foliage, with resin birch being the most susceptible. Blueberry and cloudberry were more delayed. Evergreen species continued to exhibit new injury symptoms throughout the second growing season. Foliage of Labrador tea and cranberry was still alive in the second season in areas where all deciduous foliage was dead. Primarily during the second growing season, some foliage of species outside the areas of surface flows turned brown and abscised.

An important characteristic of both spills is that no regrowth has been seen on any species which lost its foliage as a result of oil damage. This result is in direct contrast to other reported studies (Hutchinson and Freedman, 1975 and 1978; Wein and Bliss, 1973) and probably is the result of root damage as pointed out by McCown et al. (1973).

No regrowth or invasion by new individuals has occurred within areas of surface flow. Observations of an earlier (1971) CRREL crude oil spill at Fox, Alaska, showed a similar situation except that feather mosses had begun to encroach upon part of the surface flow area after six growing seasons. However, the Fox site was considerably wetter than the Poker Creek site.

The two large actual spills along TAPS did show more regrowth. The valve 7 spill, which was primarily a spray spill, showed extensive recovery of both sedge and shrub species one year after the original spill. The subarctic Steele Creek spill site was bladed by a bulldozer, while the ground was frozen, and was subsequently burned twice, in May and June, 1978. Portions of the spill were later tilled and/or fertilized but were not seeded. By September, 1978 sedges and bluejoint reedgrass (*Calamagrostis canadensis*) were regrowing in some areas of the spill (Figure 33). At both of these sites soil moisture was much higher than in the CRREL spill site. Therefore, the oil would not have penetrated so deeply into the soil so that regrowth could occur from belowground vegetative parts.

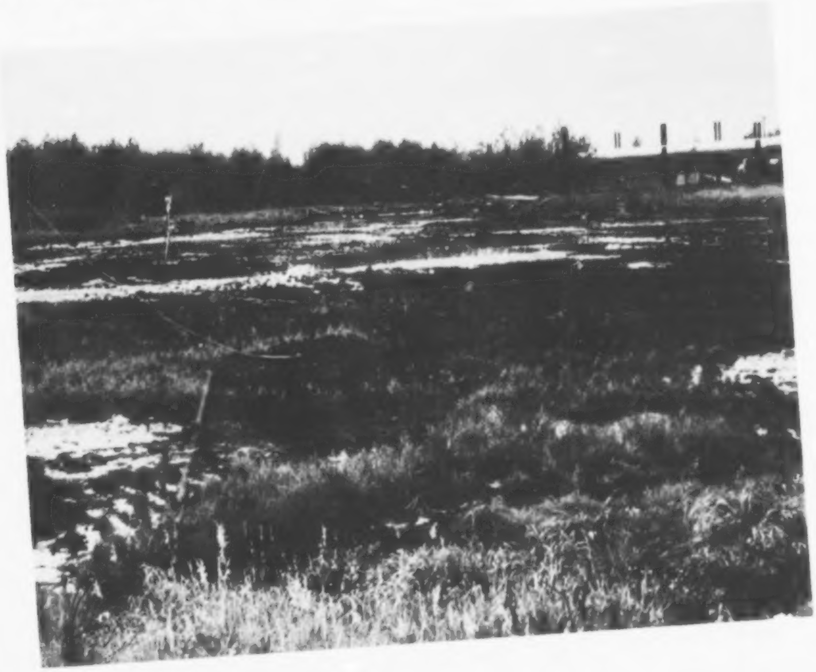


Figure 33. Steele Creek oil spill along the trans-Alaska Pipeline System. (Note regrowth of bluejoint reedgrass in the foreground.)

Others have reported that intensive point spills are less damaging than dispersed spray spills (Hutchinson and Freedman, 1978). However, the valve 7 spray spill showed good recovery probably because of low concentrations of oil per unit area.

Root respiration was reduced at all oiled sites, with the summer spill showing the greatest decrease (65%; Table 27). Studies on respiration quotients (RQ), although limited, suggest that these RQ values were raised by exposure to the crude oil in this study in contrast to arctic studies which showed decreased RQ's (Linkins and Antibus, 1978).

TABLE 27. RESPIRATION OF ECTOTROPHIC MYCORRHIZAL FEEDER ROOTS. DATA FOR O_2 AND CO_2 GIVEN AS THE MEAN OF THREE EXPERIMENTS IN μ GAS/HR G DRY WT RQ = RESPIRATORY QUOTIENT (FROM A.E. LINKINS, VPI)

Control	
O_2	219
CO_2	155
RQ	0.71
Summer (6 m downslope)	
O_2	77 (65% decrease)
CO_2	63
RQ	0.81
Summer (21 m downslope)	
O_2	163 (26% decrease)
CO_2	111
RQ	0.68
Winter (6 m downslope)	
O_2	152 (31% decrease)
CO_2	154
RQ	1.02

The results of the vegetation study are consistent with the physical and chemical changes of the oil as well as the microbial responses. The two mechanisms hypothesized for oil induced vegetation damage are direct toxicity and indirect physical disruption of plant functions by the oil. Distance downslope reduces both toxicity, since the most toxic components are volatiles which are rapidly lost, as well as physical disruption, since oil concentrations are reduced. Surface flows increase both toxic and physical effects since they lead to contact of oil of both above and below ground plant parts. However the relative importance of these two factors is not known.

Subsurface flows will make contact with roots of different species to varying extents because of differences in rooting habits. In addition, species may vary in their tolerances to contact with crude oil. There may be critical levels of oil concentrations below which plant species are undamaged. Both species' rooting habits and tolerances can influence differential susceptibility to accidental spills.

The more rapid vegetation damage on the summer spill may be due in part to a high initial concentration of volatiles. However the winter spill had higher levels of toxic components during the second and third growing seasons. Viscous, tarry oil on surface oiled areas of the winter spill reduced infiltration and may have decreased soil moisture. These factors may be responsible for the delayed mortality of the black spruce on the winter spill. By the end of the third growing season, the winter spill with its 250% greater area of surface flow may have more total vegetation damage than the summer spill because of these differences.

Both the nature of injury and the lack of vegetative recovery have important implications for restoration following oil spills. The lack of reinvasion by seedlings may be due to the hydrophobic surface of the oil-soaked moss layer (Deneke et al., 1975; McGill, 1977). However, even if the hydrophobic surface were disrupted to allow for germination as suggested by McGill (1977) the problem of toxic components of oil within the soil might persist for a number of years. In addition, restorative measures which disrupt the surface organic mat on a permafrost site pose a threat to the thermal stability of the area.

Conclusions

The response of a subarctic permafrost community to a massive introduction of crude oil is represented in Figure 34.

Crude petroleum spilled onto subarctic permafrost slopes penetrates the moss layer. During periods of thaw the oil moves into and saturates lower organic layers of the soil. The oil then spreads downslope and largely follows drainage channels; this spread is concealed by the moss cover. Thus the area actually contaminated is always larger (2.5 to 10 times) than the area where oil is readily visible on the surface. Topography will have a significant effect upon the amount of land contaminated.

In winter, snow cover reduces the initial extent of areal spread, but such cover also provides additional concealment as warm oil can penetrate the snow and move along and into the underlying moss and organic soil layers.

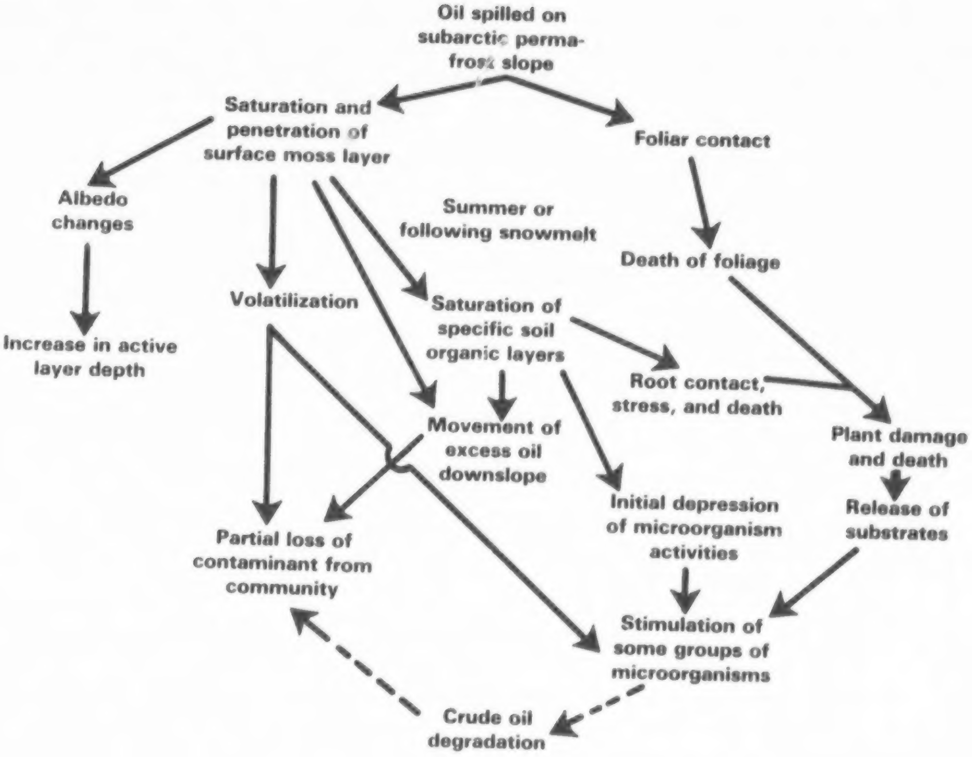

Oil spilled on subarctic permafrost slope
Saturation and penetration of surface moss layer
Foliar contact
Albedo changes
Summer or following snowmelt
Death of foliage
Volatilization
Saturation of specific soil organic layers
Increase in active layer depth
Root contact, stress, and death
Movement of excess oil downslope
Plant damage and death
Initial depression of microorganism activities
Release of substrates
Partial loss of contaminant from community
Stimulation of some groups of microorganisms
Crude oil degradation

Figure 34. Flowchart representing the observed effects of a massive crude oil spill on a subarctic permafrost site. These responses have been observed during the first three growing seasons after the oil spill. Dashed lines indicate reactions that have not been confirmed.

Oil introduction into this ecosystem represents a strong perturbation. Major changes in microorganism populations are evident. Foliage immediately in contact with the contaminant is killed and slow death can occur to plants that have roots exposed to the moving underground oil mass. Some species appear resistant to the effects of crude oil in the soil.

Crude oil contaminated areas in subarctic regions may not be stabilized even several growing seasons after the spill. Death of plants, microbial changes, oil compositional changes, and increases in the active layer may continue for an undetermined time period.

Although volatiles in crude oil near the soil surface are lost rapidly after a spill, in the lower soil horizons some of even the most volatile components of the crude can remain for some time. No evidence for the rapid biological degradation of the oil has been obtained. In addition, no evidence of recovery by seedling establishment on oil contaminated surfaces has been observed during the first three growing seasons after the spill.

It appears that a crude oil contaminanation will be damaging, although not catastrophic to a subarctic community for many decades.

Recommendations

Based on the results obtained in this three year study, the following recommendations are made:

1) Consideration should be given to a "no clean-up strategy" for small spills in subarctic permafrost areas (a few thousand gallons or less), where no surface water course is involved and ready access is not available. Providing access to a site can be more destructive of sensitive permafrost areas than the spilled oil. In any case, the size of the spill should be considered since very large spills could have much greater impact. Also the type of spill and product spilled will have varying effects, however, these were not a consideration in this study.

2) Reaction to large summer oil spills needs to be rapid to minimize the area impacted in the incident since the oil will continue to move downslope for weeks following spillage. Reaction to winter spills can be delayed since the oil will initially impact a much smaller area and downslope flow will quickly stop as the oil cools. Clean-up operations should be completed before snow melt when the oil may remobilize and impact additional unaffected areas.

3) Research at the present site needs to be continued in order to determine long-term changes. The uniqueness of the site in terms of background information and size of the spills should be fully utilized for future studies.

4) Long term changes in chemical composition of the oil and the rate of encroachment by native species of vegetation onto oil saturated areas need to be determined.

5) The environmental factor(s) limiting oil degradation (e.g. temperature, moisture, nutrient availability, aeration, etc.) needs to be documented in order to devise appropriate restorative measures and to assess the importance of such environmental factors for natural recovery. For example, soil moisture data in this study indicate reduced moisture in heavily oil contaminated soil but aeration has not been measured.

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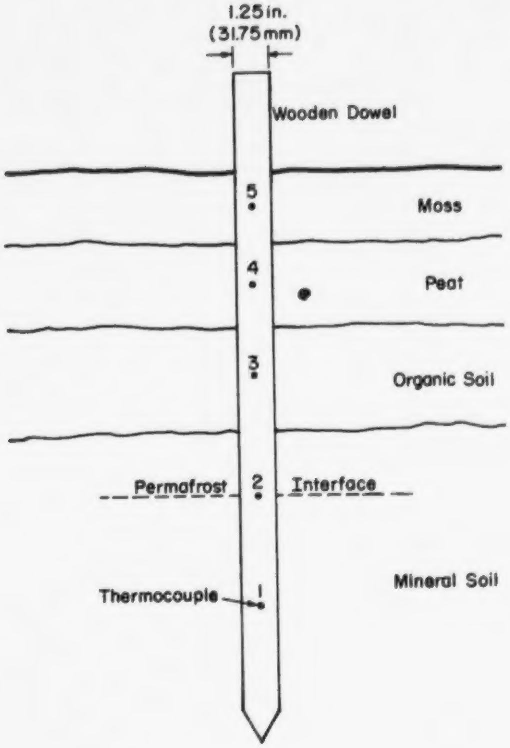
APPENDIX


1.25 in.
(31.75 mm)
Wooden Dowel
5
Moss
4
Peat
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Organic Soil
Permafrost
2
Interface
Mineral Soil
Thermocouple
1

Figure A-1. Vertical Thermocouple Assembly

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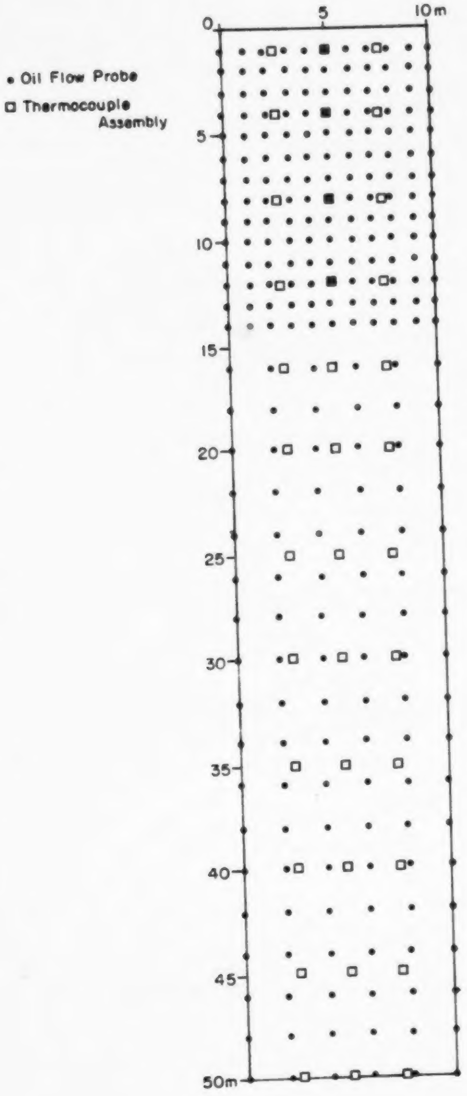
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• Oil Flow Probe
□ Thermocouple Assembly
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40
45
50m

Figure A-2. Spill Sites.

TABLE A-1. AVERAGE SOIL TEMPERATURES

(for first 10 meters downslope)

DATE	Thermocouple LEVEL *	WINTER SPILL	SUMMER SPILL	CONTROL
6/22/76	5.	12.7°C	8.8	10.1
	4.	3.6	3.45	2.0
	3.	0.5	0.1	- 0.1
	2.	- 0.1	-0.35	- 0.4
	1.	- 0.5	-0.6	- 0.5
7/20/76	5.	22.5	14.3	12.7
	4.	6.6	6.3	3.6
	3.	2.2	2.2	1.05
	2.	1.3	0.7	0.15
	1.	0.4	0.35	- 0.2
8/12/76	5.	13.2	12.1	11.2
	4.	6.3	6.5	4.0
	3.	2.4	2.8	1.55
	2.	1.85	1.2	0.35
	1.	0.7	0.3	- 0.2
9/28/76	5.	6.05	5.9	4.9
	4.	2.5	2.25	1.3
	3.	0.95	0.7	0.2
	2.	0.5	0.4	- 0.1
	1.	0.45	-0.1	- 0.2
6/2/77	5.	17.4	14.4	11.4
	4.	6.2	4.7	1.7
	3.	- 0.3	0.5	- 0.05
	2.	- 0.4	0.15	- 0.2
	1.	- 0.55	-0.2	- 0.3
8/16/77	5.	17.25	21.1	13.15
	4.	7.0	10.6	5.0
	3.	3.3	4.85	2.0
	2.	2.4	2.5	0.7
	1.	1.1	1.3	- 0.4
7/12/78	5.	23.4	24.5	13.6
	4.	10.15	15.85	5.7
	3.	5.0	7.15	2.15
	2.	3.5	2.8	0.3
	1.	1.6	1.5	0.0

*See Figure Al.

TABLE B-1. RESULTS FROM SILICA GEL/ALUMINA FRACTIONATION OF OILY SOIL EXTRACTS FROM CARIBOU-POKER CREEKS

	Major Oil components (%)				
SAMPLE	Alkanes	Aromatics	Asphalfenes	sol.	insol.
Winter Min 7/15/76	30.4	29.4	16.4	8.9	14.8
Summer Min 7/15/76	22.9	20.4	34.8	13.6	8.3
Control Min 7/15/76	0.9	1.3	92.8	7.2	0
Winter Organic 7/15/76	32.9	30.0	11.7	8.7	16.7
Summer Organic 7/15/76	33.0	29.5	13.9	8.1	15.4
Control Organic 7/15/76	0.7	2.4	88.5	12.0	0
Winter Moss 7/15/76	42.1	34.4	8.4	9.5	5.6
Summer Moss 7/15/76	37.6	36.6	7.4	12.8	5.6
Control Moss 7/15/76	1.1	0.7	87.0	17.9	0
Winter Min 9/17/76	41.1	31.8	10.6	8.8	7.8
Summer Min 9/17/76	27.6	26.4	18.8	10.4	16.6
Control Min 9/17/76	0.5	1.0	98.0	11.3	0
Winter Organic 9/17/76	42.1	33.1	8.0	8.7	8.1
Summer Organic 9/17/76	33.8	32.3	14.8	8.8	10.4
Control Organic 9/17/76	0.2	0.7	87.8	12.3	0
Winter Moss 9/17/76	40.0	32.4	11.6	9.5	6.4
Summer Moss 9/17/76	40.4	36.0	8.4	8.8	6.4
Winter Organic 3/29/77	36.6	34.9	10.7	9.5	8.2
Summer Organic 3/29/77	48.9	35.9	8.8	8.2	0
Control Organic 3/29/77	0.1	0.8	93.0	6.5	0

TABLE B-1 (Cont'd)

	Major Oil components (%)				
SAMPLE	Alkanes	Aromatics	Asphalfenes	sol.	insol.
Winter Moss 3/29/77	44.9	35.9	10.3	8.4	0.4
Summer Moss 3/29/77	36.8	37.2	10.0	8.9	7.0
Winter Min 4/5/77	32.2	29.7	16.7	12.3	9.1
Summer Min 4/5/77	30.2	30.8	17.1	10.8	11.1
Winter Organic 4/5/77	37.3	29.0	-	9.8	-
Summer Organic 4/5/77	32.6	32.5	12.2	13.3	9.4
Winter Moss 4/5/77	36.8	28.7	-	10.6	-
Winter Min 7/13/77	31.6	30.5	15.7	11.6	10.7
Winter Organic 7/13/77	38.2	32.6	11.0	10.7	7.5
Summer Organic 7/13/77	36.0	34.5	8.0	11.0	10.4
Winter Moss 7/13/77	34.4	34.1	10.8	11.0	9.7
Summer Moss 7/13/77	33.1	32.6	12.6	11.9	9.8
Winter Min 8/1/77	29.3	25.0	22.9	12.0	10.8
Summer Min 8/1/77	19.2	24.8	40.2	12.2	3.6
Winter Organic 8/1/77	30.6	28.0	19.6	12.4	9.4
Summer Organic 8/1/77	30.5	26.8	20.1	13.0	9.6
Summer Organic 1/5/78	32.8	37.6	10.6	10.2	8.7
Winter Moss 1/5/78	36.3	43.4	9.7	9.9	0.7
Summer Moss 1/5/78	33.4	35.4	9.2	10.9	11.1
Control Moss 1/5/78	0	6.9	59.7	58.3	0
Winter Min 6/27/78	33.9	30.6	16.1	10.4	9.2
Summer Min 6/27/78	26.6	24.2	25.0	10.0	14.3
Winter Organic 6/27/78	42.0	32.6	6.3	9.3	9.9
Summer Organic 6/27/78	34.4	32.6	8.6	10.0	14.4

TABLE B-2. RESULTS FROM SILICA GEL/ALUMINA FRACTIONATION OF SAMPLES FROM CARIBOU-POKER (7/27/78)

	Major Oil components (%)				
				NSO	
SAMPLE	Alkanes	Aromatics	Asphalfenes	sol.	insol.
S 4-5, 8-9 Moss	40.8	38.3	2.1	7.1	11.8
S 1-2, 8-9 Moss	29.7	29.4	18.7	8.5	13.7
S 7, 34 Moss	35.0	36.5	6.0	7.5	15.0
S 4-5, 8-9 Organic	39.6	33.0	12.2	8.5	6.8
S 7, 34 Organic	40.7	32.1	8.6	8.9	9.7
S 1-2, 8-9 Organic	39.0	32.4	12.2	8.7	7.6
S 1-2, 8-9 Min	23.9	26.7	30.5	12.1	6.9
S 4-5, 8-9 Min	31.1	33.1	14.9	9.6	11.4
W 8-9, 11-12 Moss	35.0	36.5	6.0	8.4	14.1
W 8-9, 11-12 Organic	35.2	35.7	9.3	9.0	10.9
W 15, 34 Organic	32.3	31.9	11.9	10.4	13.5
W 8-9, 11, 12 Min	35.2	32.8	15.3	6.3	10.4

TABLE B-3. RESULTS FROM FRACTIONATION OF SAMPLES FROM OTHER ALASKAN SPILL AREAS

			MAJOR OIL COMPONENTS (%)			NSO	
LOCATION	SPILL DATE	SAMPLE DATE	Alkanes	Aromatics	Asphaltenes	Sol.	Insol.
Barrow 313	1970	July 1977	22.6	26.8	20.6	14.0	15.9
Barrow 311	1970	July 1977	34.4	34.3	10.8	10.9	9.5
Barrow 152 D	1971	July 1977	31.1	32.5	12.1	12.4	11.8
Fox	1971	July 1976	32.4	35.1	10.8	10.9	10.9
Prudhoe Oil (orig. oil used for spill)	July 1976	-	35.2	36.8	8.4	12.1	7.5
Prudhoe 2CR	1976	July 1977	37.5	35.5	7.1	10.9	8.9
Prudhoe 5 CR	1976	July 1977	36.1	37.2	7.7	9.8	9.2

TABLE B-4. OIL IN SOIL EXTRACTS (%)

	Mineral			Organic			Moss		
SAMPLE DATE	WIN.	SUM.	CON.	WIN.	SUM.	CON.	WIN.	SUM.	CON.
7/15/76	9.81	1.65	0.73	89.0	57.6	1.35	266	207	2.49
9/17/76	6.52	3.85	0.45	33.5	7.09	2.03	249	211	0.91
3/29/77	-	-	-	24.1	181	2.29	255	147	2.12
4/5/77	4.03	3.38	0.10	115	26.9	0.76	137	-	0.82
7/13/77	4.70	3.00	0.10	190	283	0.70	234	194	1.90
8/1/77	3.6	1.5	0.2	7.3	12.4	0.6	-	-	-
1/5/78	-	-	-	-	50.1	-	183	69.3	-
6/27/78	3.89	1.76	-	16.8	31.1	-	-	-	-

TABLE B-5. SOIL pH

	Mineral			Organic		
SAMPLE DATE	WIN.	SUM.	CON.	WIN.	SUM.	CON.
7/15/76	-	-	-	3.4	3.5	3.5
9/17/76	3.6	3.5	3.6	3.3	3.5	3.2
3/29/77	-	-	-	3.5	3.4	3.5
4/5/77	3.9	4.0	3.9	3.9	3.8	3.8
7/13/77	-	-	-	-	-	3.7

TABLE B-6. EXCHANGEABLE CATIONS IN SOIL SAMPLES (meq/100 g)

SAMPLE	Na^+	K^+	Ca^{++}	Mg^{++}	NH_4^+
Winter Mineral 7/15/76	0.09	0.35	12.7	2.61	0.035
Summer Mineral 7/15/76	0.10	0.37	15.1	3.25	0.028
Control Mineral 7/15/76	0.09	0.24	18.5	3.18	0.044
Winter Organic 7/15/76	0.26	1.38	24.0	4.48	0.046
Summer Organic 7/15/76	0.32	1.48	89.2	13.5	0.051
Control Organic 7/15/76	0.19	1.20	43.6	7.74	0.034
Winter Mineral 9/17/76	0.07	0.14	4.3	0.90	0.065
Summer Mineral 9/17/76	0.07	0.15	6.5	1.91	0.081
Control Mineral 9/17/76	0.06	0.09	3.6	1.04	0.064
Winter Organic 9/17/76	0.10	0.33	8.4	2.86	0.073
Summer Organic 9/17/76	0.12	0.29	13.9	3.75	0.077
Control Organic 9/17/76	0.19	0.60	16.4	4.78	0.061
Winter Organic 3/29/77	-	-	-	-	0.082
Summer Organic 3/29/77	-	-	-	-	0.010
Control Organic 3/29/77	-	-	-	-	0.060

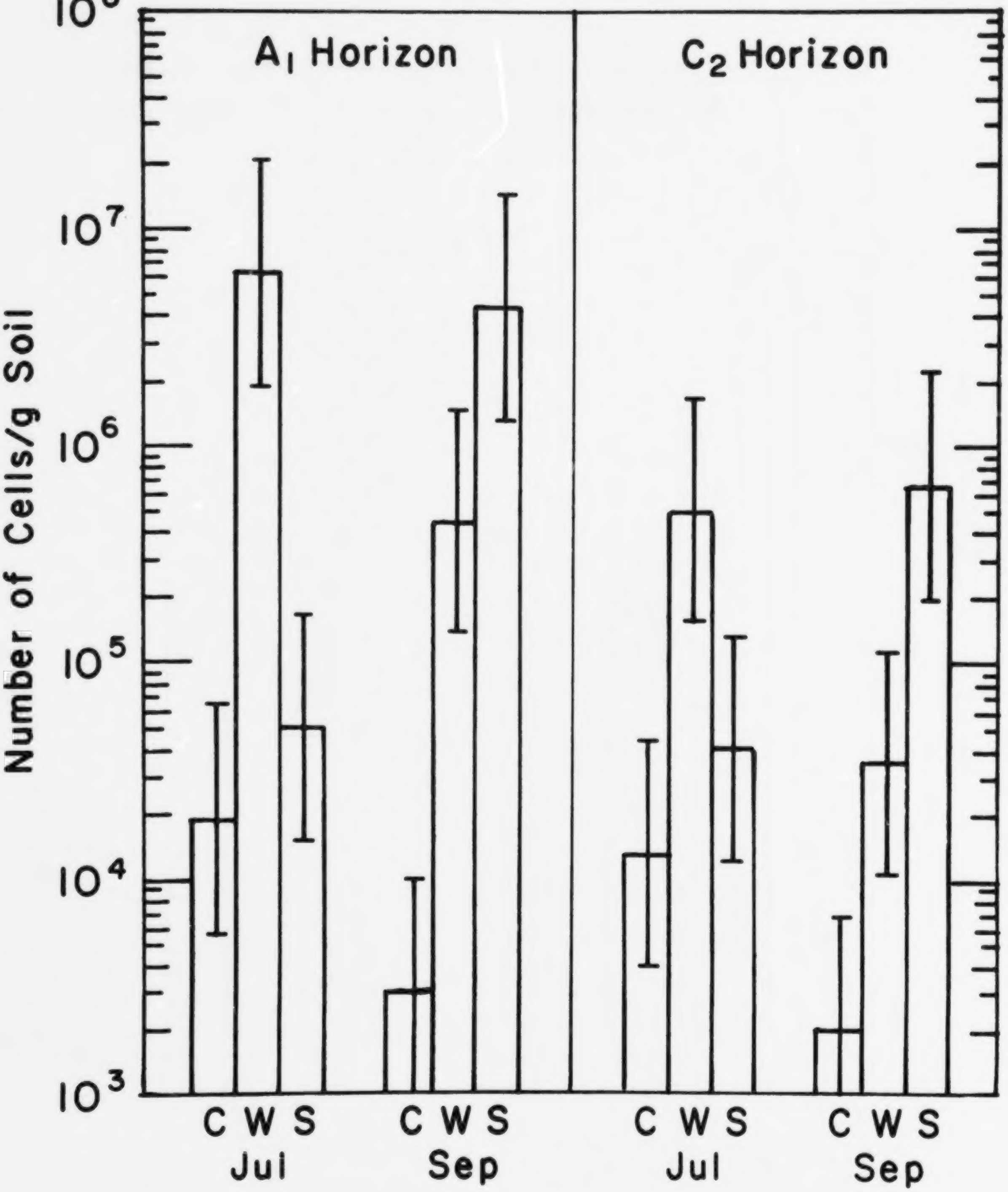

Figure C-1. 1976 MPN counts (20°C) of denitrifying bacteria in oiled and unoiled (control plots. C = Control Plot; W = Winter Plot; S = Summer Plot; Brackets represent 95% confidence interval.

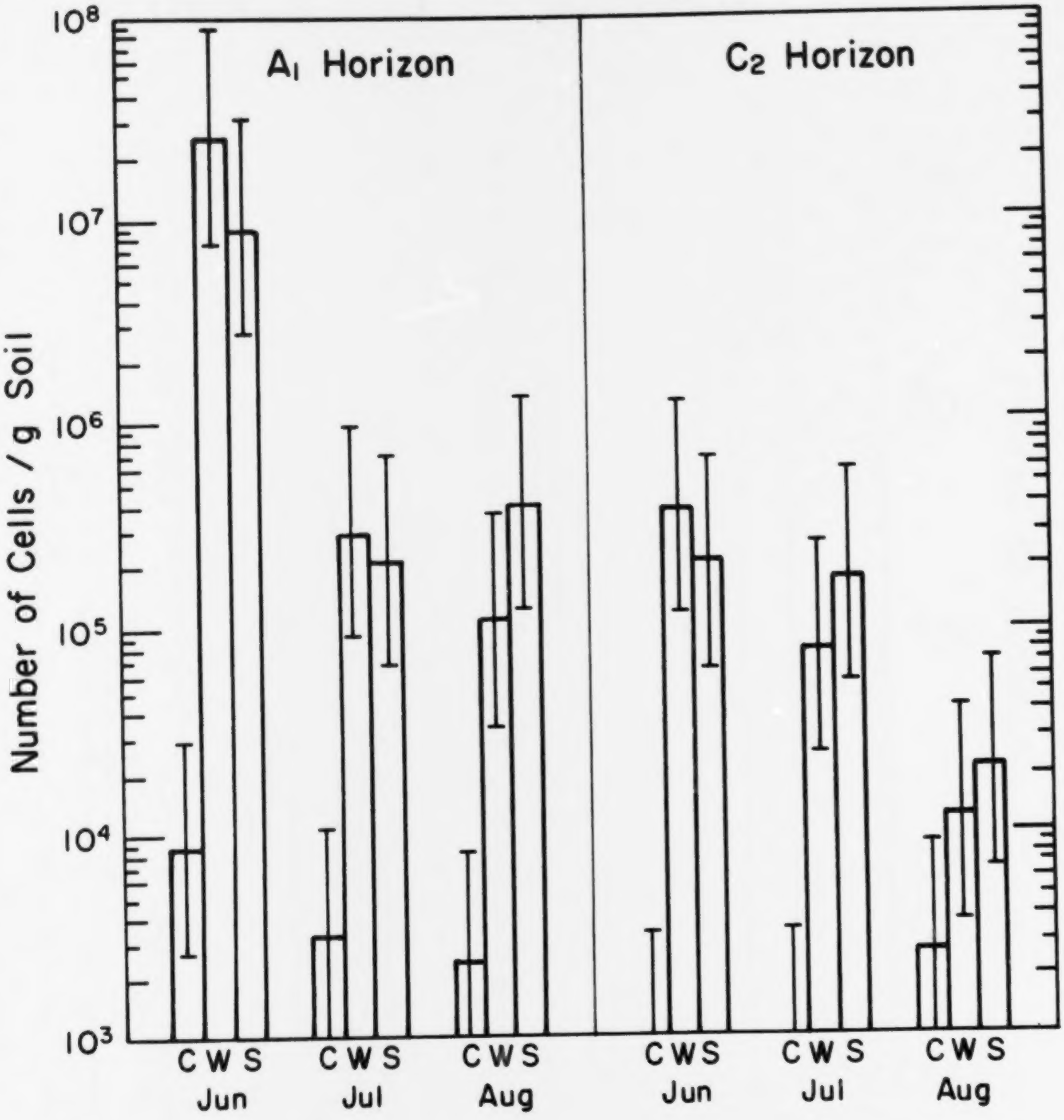

Figure C-2. 1977 MPN counts (20°C) of denitrifying bacteria in oiled and unoiled (control) plots. C = Control Plot; W = Winter Plot; S = Summer Plot; Brackets represent 95% confidence interval.

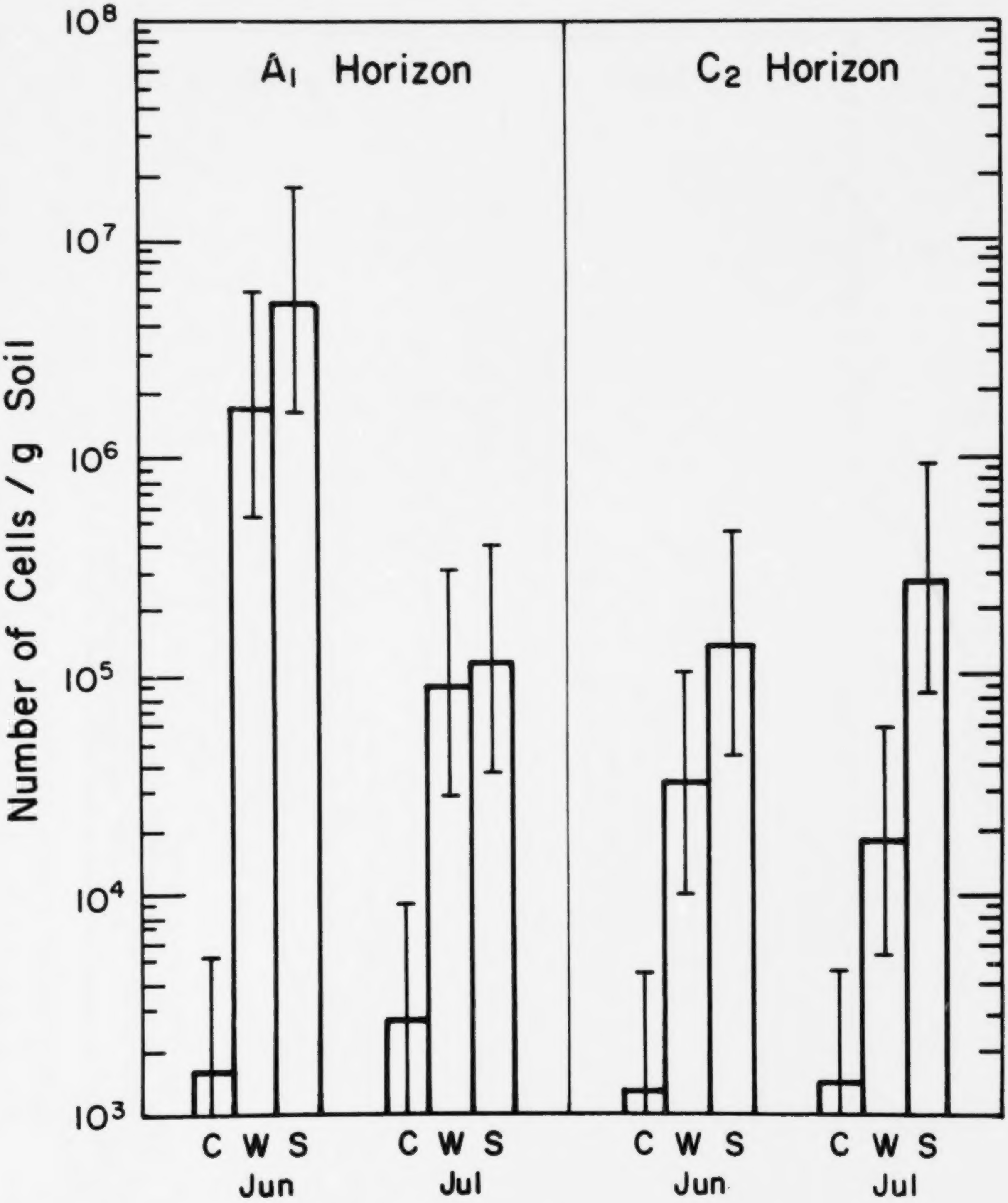

Figure C-3. 1978 MPN counts (20°C) of denitrifying bacteria in oiled and unoiled (control) plots. C = Control Plot; W = Winter Plot; S = Summer Plot; Brackets represent 95% confidence interval.

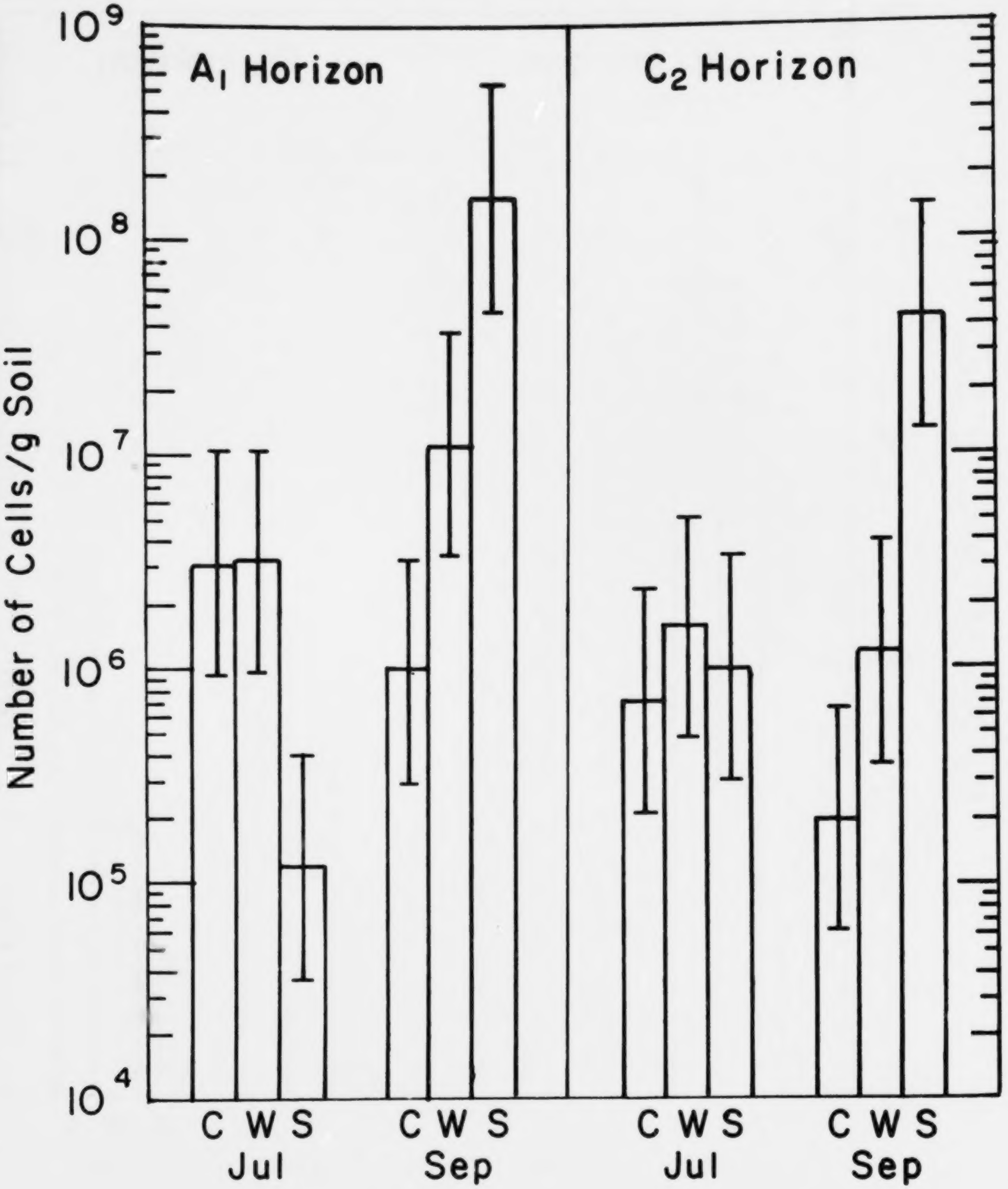

Figure C-4. 1976 MPN counts (20°C) of cellulose-utilizing fungi in oiled and unoiled (control) plots. C = Control Plot; W = Winter Plot; S = Summer Plot; Brackets represent 95% confidence interval.

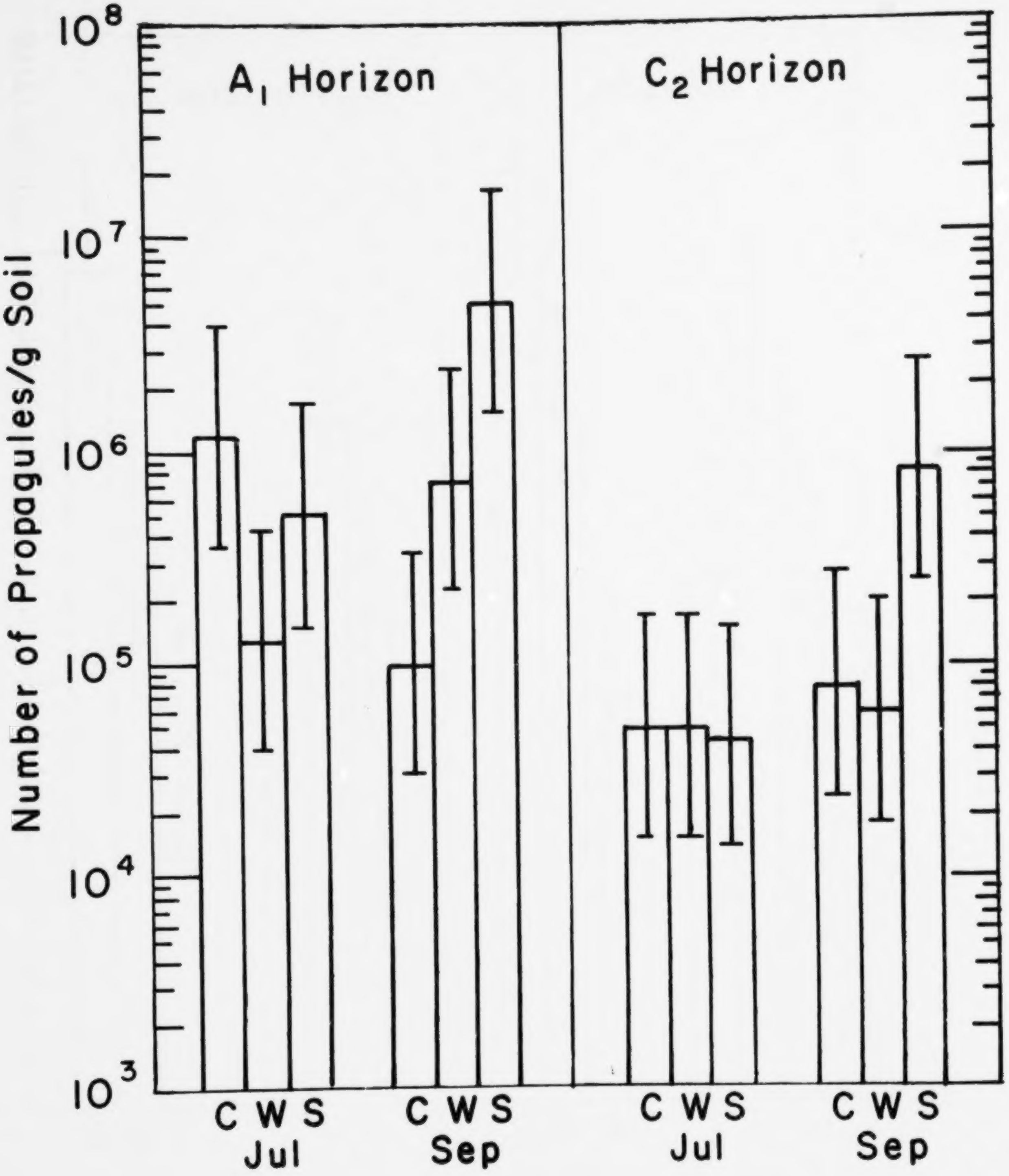

Figure C-5. 1976 MPN counts (20°C) of cellulose-utilizing fungi in oiled and unoiled (control) plots. C = Control Plot; W = Winter Plot; S = Summer Plot; Brackets represent 95% confidence interval.

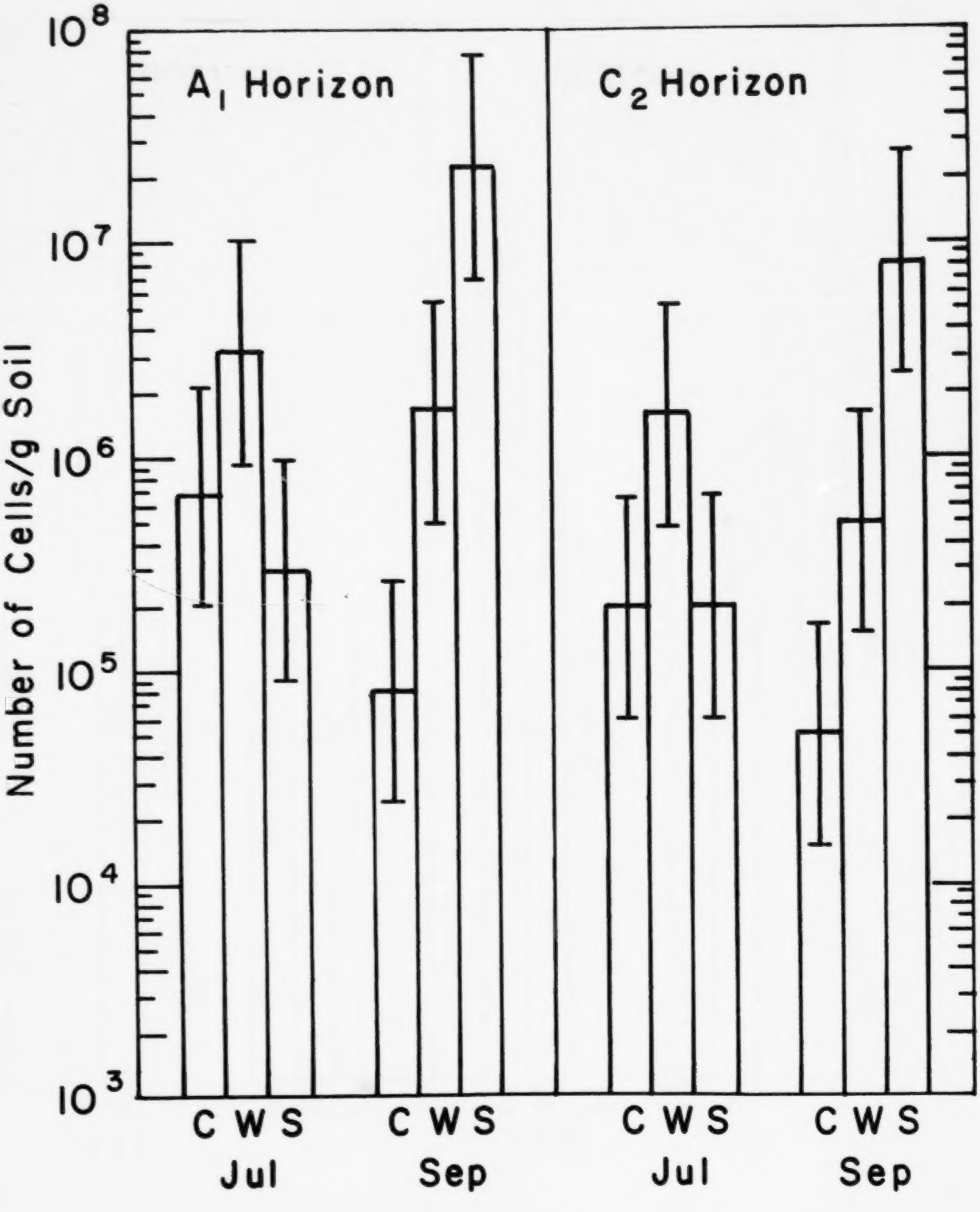

Figure C-6. 1976 MPN counts (20°C) of oil utilizing-bacteria in oiled and unoiled (control) plots. C = Control Plot; W = Winter Plot; S = Summer Plot; Brackets represent 95% confidence interval.

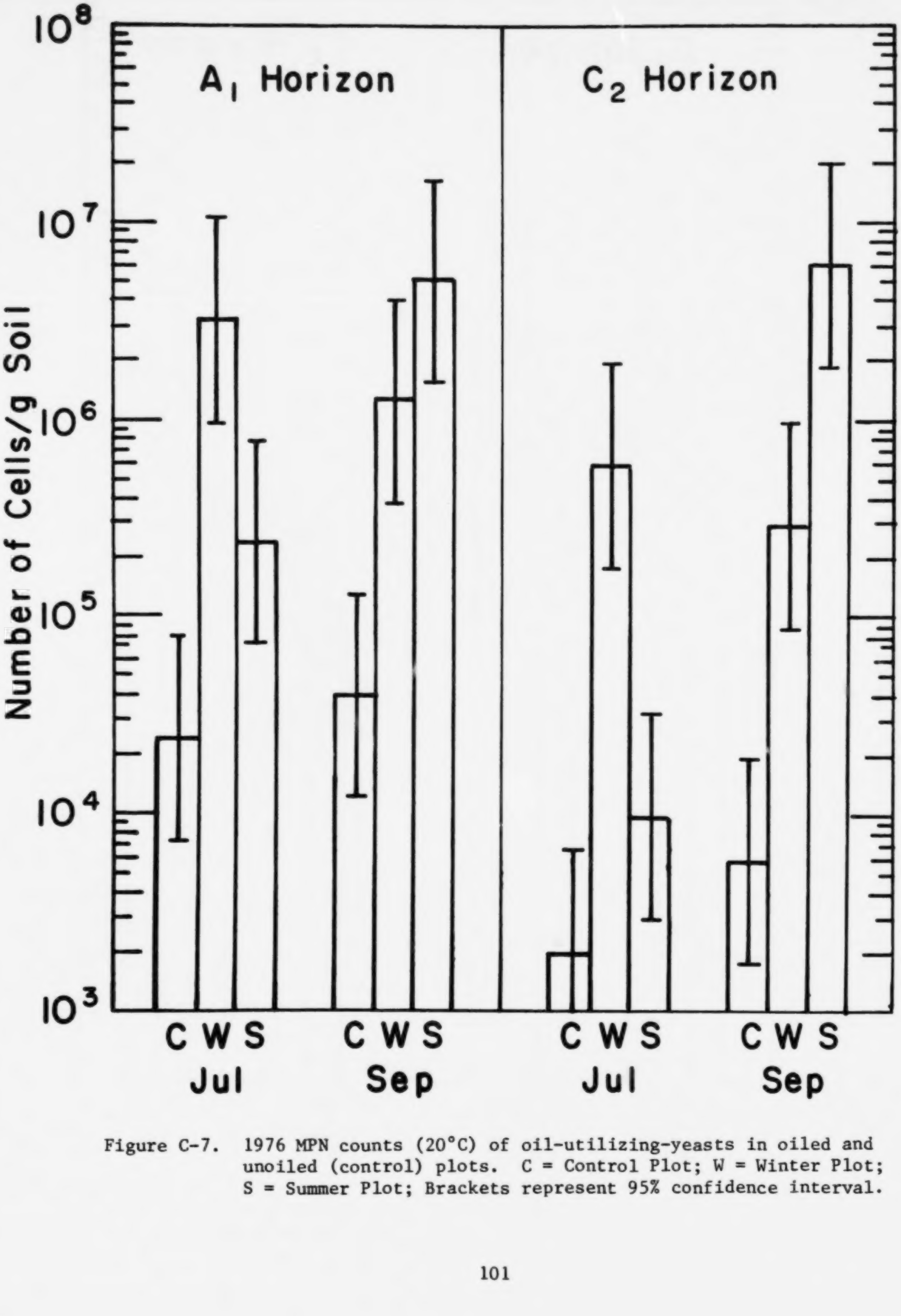

Figure C-7. 1976 MPN counts (20°C) of oil-utilizing-yeasts in oiled and unoiled (control) plots. C = Control Plot; W = Winter Plot; S = Summer Plot; Brackets represent 95% confidence interval.

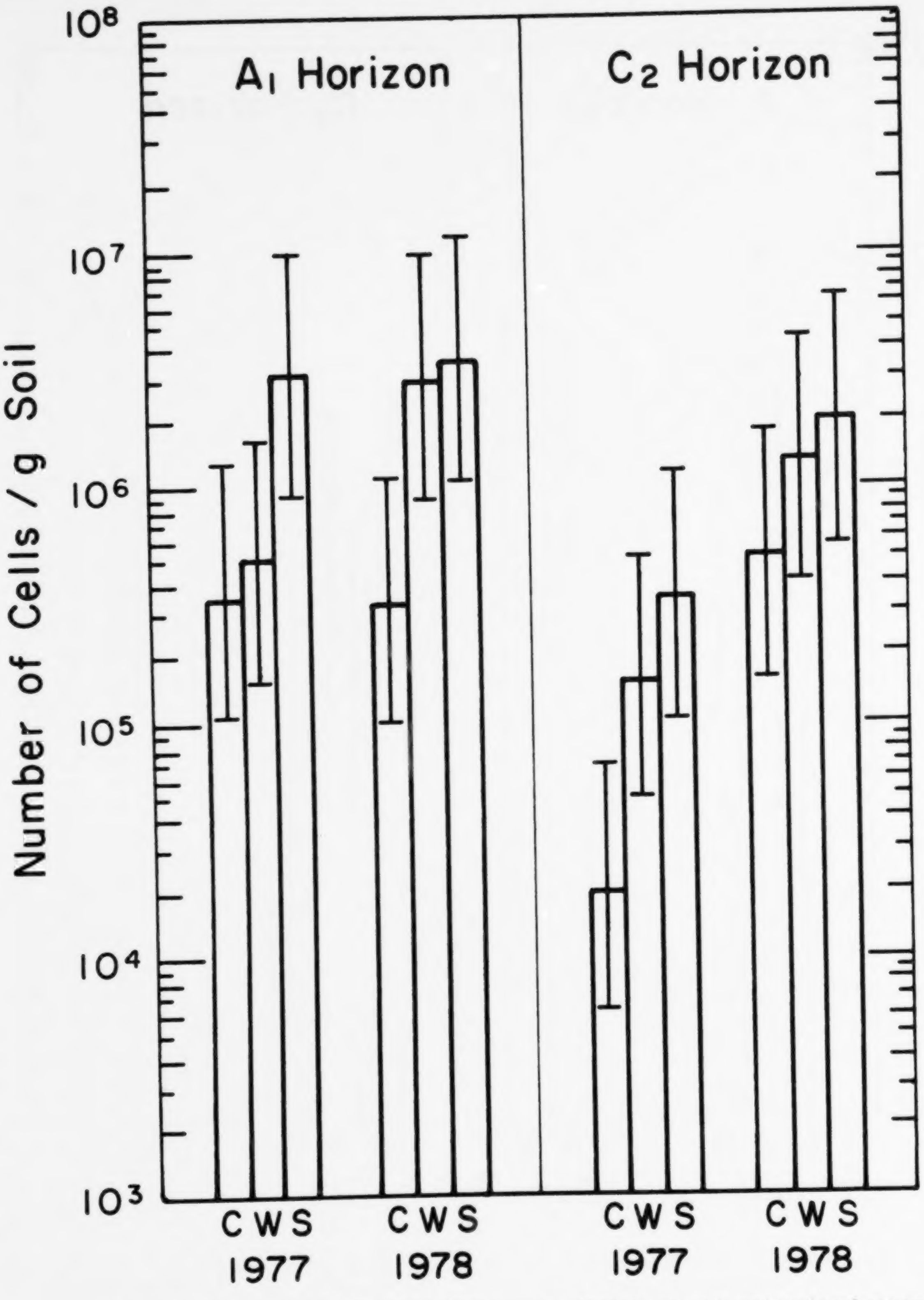

Figure C-8. 1977 and 1978 MPN counts (20°C) of oil-utilizing-bacteria in oiled and unoiled (control) plots. C = Control Plot; W = Winter Plot; S = Summer Plot; Brackets represent 95% confidence interval.

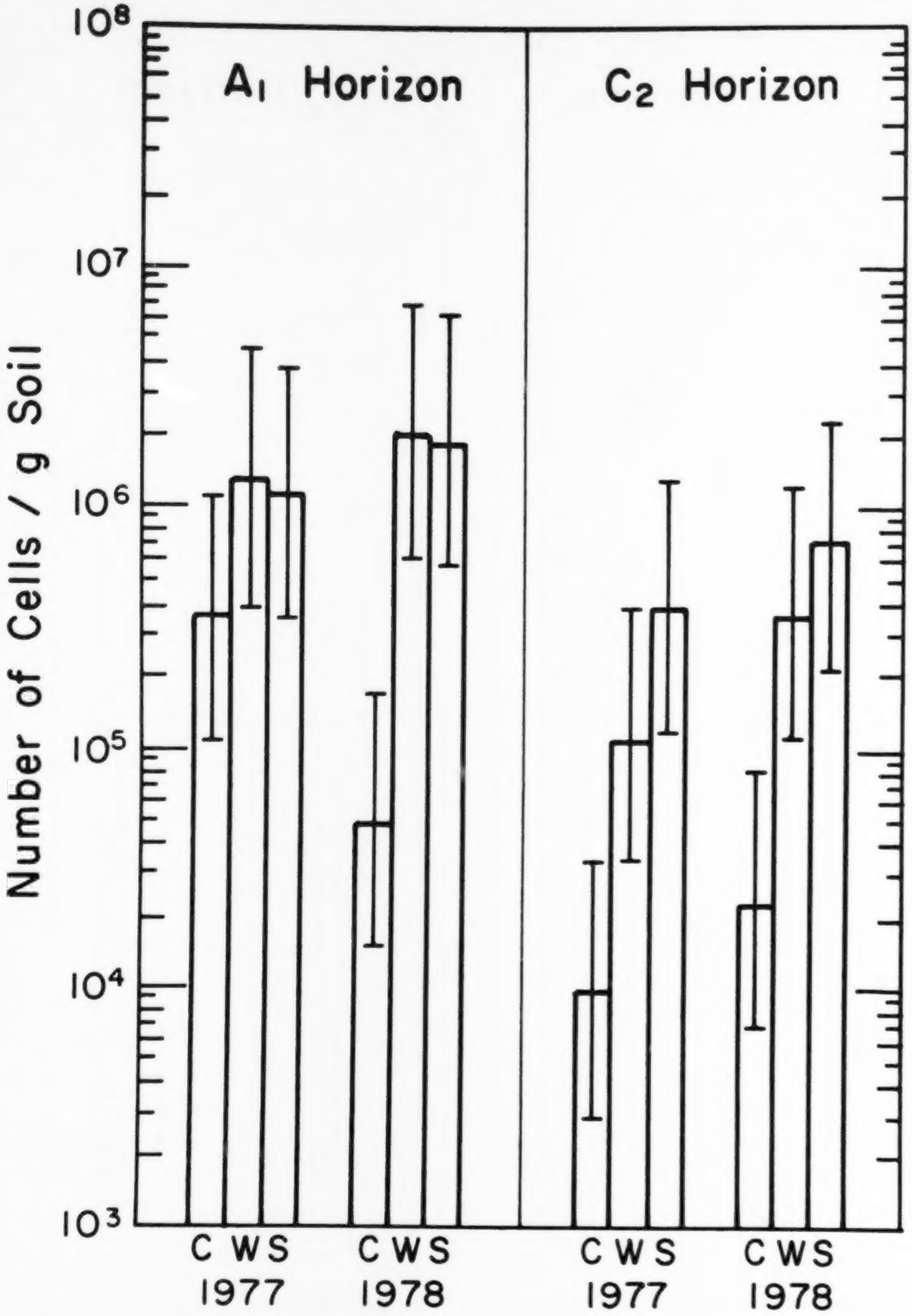

Figure C-9. 1977 and 1978 MPN counts (20°C) of oil-utilizing-yeasts in oiled and unoiled (control) plots. C = Control Plot; W = Winter Plot; S = Summer Plot; Brackets represent 95% confidence interval.

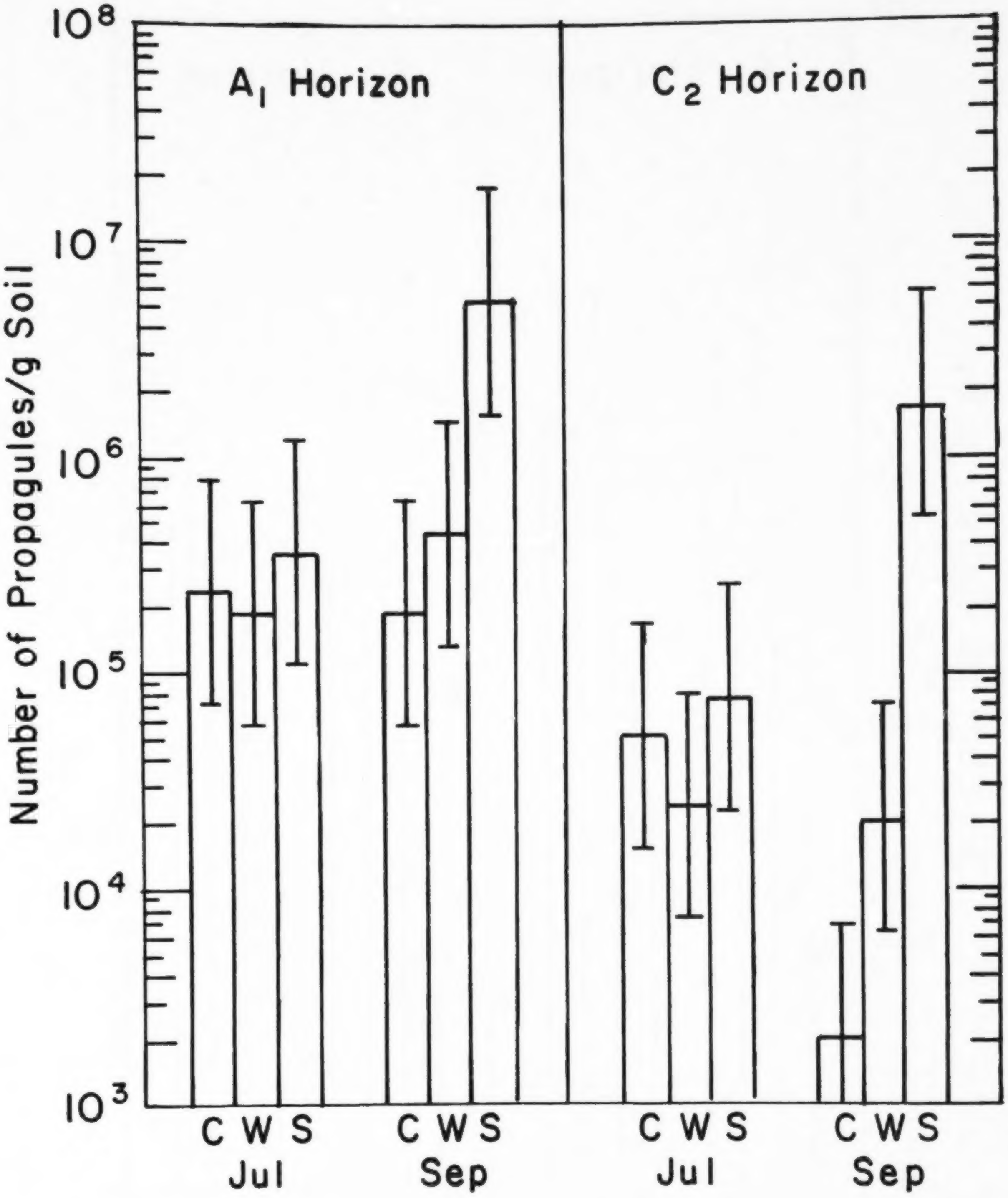

Figure C-10. 1976 MPN counts (20°C) of oil-utilizing fungi in oiled and unoiled (control) plots. C = Control Plot; W = Winter Plot; S = Summer Plot; Brackets represent 95% confidence interval.

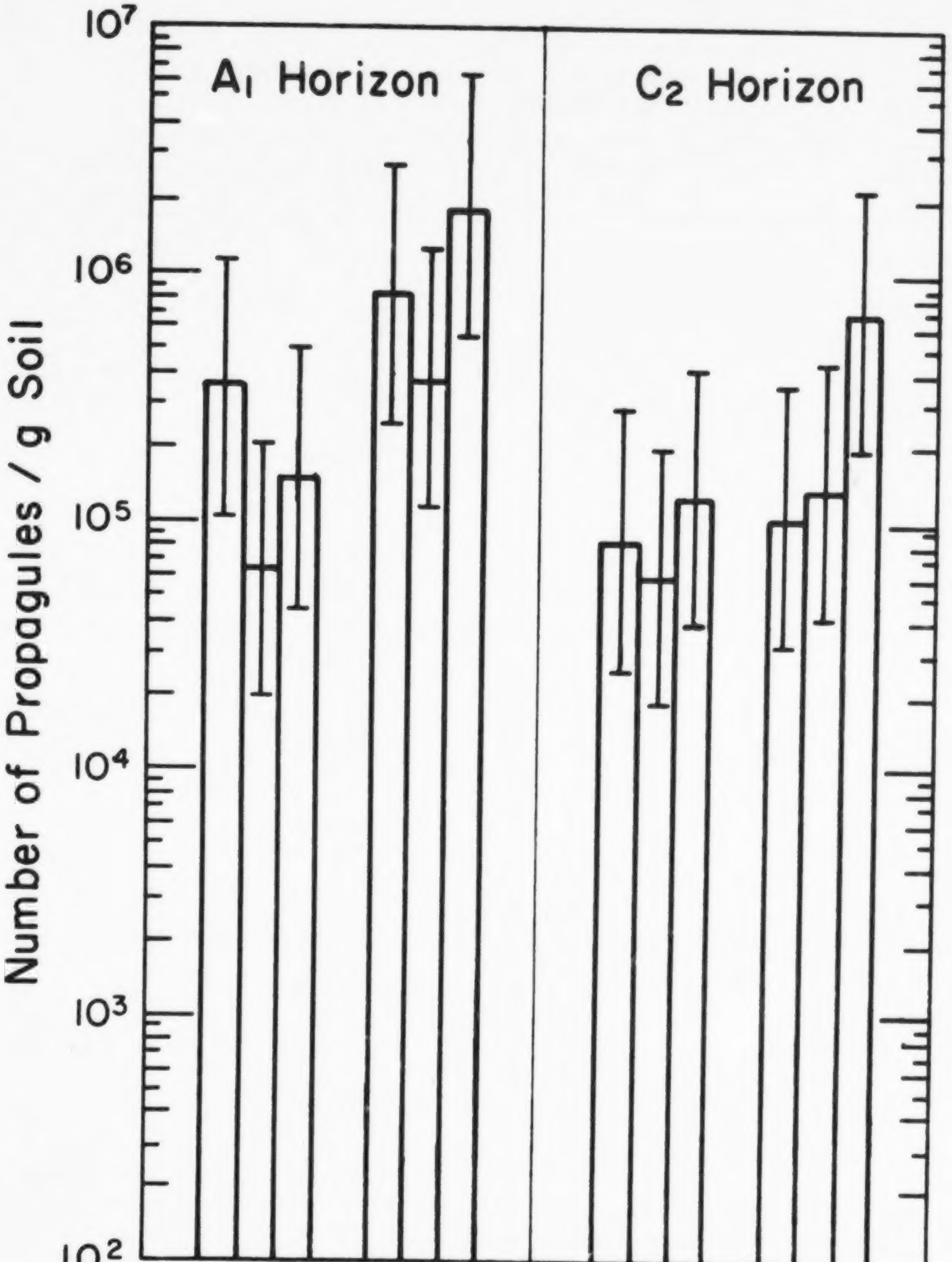

Figure C-11. 1977 and 1978 MPN counts (20°C) of oil-utilizing fungi in oiled and unoiled (control) plots. C = Control Plot; W = Winter Plot; S = Summer Plot; Brackets represent 95% confidence interval.

TABLE C-1. 20°C HETEROTROPHIC BACTERIAL COUNTS (x 10^6/g soil) IN OILED AND UNOILED (CONTROL) PLOTS

Sampling time	Control plot	Winter plot	Summer plot
1976		A_1 Horizon	
February[a]	12	11	-
June	27	300**	-
July[b]	28	440**	18*cc
August	16	280**	570**cc
September	13	260**	1300**cc
		C_2 Horizon	
February[a]	7	5**	-
June	11	8	-
July[b]	9	42**	7cc
August	8	24**	28**
September	3	19*	280**cc
1977		A_1 Horizon	
June	29	720**	1800**cc
July	18	330**	1200**cc
August	22	180**	880**cc
		C_2 Horizon	
June	6	44**	280**cc
July	4	94**	170**cc
August	2	55*	150**cc
1978		A_1 Horizon	
June	24	370**	700**cc
July	25	380**	370**
		C_2 Horizon	
June	10	72**	71**
July	6	56**	34**cc

[a] Within 24 hours after the winter oil spill.
[b] Within 24 hours after the summer oil spill.
* Significantly different from the control at 5% level.
** Significantly different from the control at 1% level.
cc Significantly different from the winter at 1% level.

TABLE C-2. 20°C FILAMENTOUS FUNGAL PROPAGULE COUNTS (× 10^4/g soil) IN OILED AND UNOILED (CONTROL) PLOTS

Sampling time	Control plot	Winter plot	Summer plot
1976		A_1 Horizon	
February[a]	100	160	-
June	230	15**	-
July[b]	160	27**	60**cc
August	110	80*	75**
September	62	72	460**cc
		C_2 Horizon	
February[a]	25	8**	-
June	33	3**	-
July[b]	13	n.d.	35**
August	20	5**	11**c
September	7	3**	91**cc
1977		A_1 Horizon	
June	270	47**	300cc
July	170	41**	210*cc
August	150	21**	180*cc
		C_2 Horizon	
June	35	20**	29**cc
July	10	8	20**cc
August	22	9**	18**cc
1978		A_1 Horizon	
June	170	420**	470**
July	160	500**	420**cc
		C_2 Horizon	
June	25	26	n.d.
July	26	34**	37**

a Within 24 hours after the winter oil spill.
b Within 24 hours after the summer oil spill.
n.d. Not determined.
* Significantly different from the control at 5% level.
** Significantly different from the control at 1% level.
c Significantly different from the winter at 5% level.
cc Significantly different from the winter at 1% level.

TABLE C-3. 20°C YEAST COUNTS ($\times 10^5$/g soil) IN OILED AND UNOILED (CONTROL) PLOTS

Sampling time	Control plot	Winter plot	Summer plot
1976		A_1 Horizon	
February[a]	5.4	7.2	-
June	8.1	790**	-
July[b]	2.7	120**	5cc
August	7.0	340**	1500**cc
September	11	220**	1800**cc
		C_2 Horizon	
February[a]	1.2	1.2	-
June	1.0	8.6**	-
July[b]	0.6	7.4**	0.6cc
August	1.4	25**	110**cc
September	1.1	22**	590**cc
1977		A_1 Horizon	
June	3.1	210**	3100**cc
July	5.1	330**	5200**cc
August	4.0	250**	2900**cc
		C_2 Horizon	
June	0.7	53**	930**cc
July	0.2	53**	400**cc
August	1.2	48**	500**cc
1978		A_1 Horizon	
June	14	3100**	3400**
July	12	3200**	2400**cc
		C_2 Horizon	
June	1	410**	390**
July	2	280**	160**cc

[a] Within 24 hours after the winter oil spill.
[b] Within 24 hours after the summer oil spill.
** Significantly different from the control at 1% level.
cc Significantly different from the winter at 1% level.

TABLE C-4. 20°C PROTEOLYTIC BACTERIAL COUNTS (x 10^5/g soil) IN OILED AND UNOILED (CONTROL) PLOTS IN 1976

Sampling time	Control plot	Winter plot	Summer plot
		A_1 Horizon	
February[a]	67	60	-
June	200	1300**	-
July[b]	68	800**	110**cc
August	34	150**	3900**cc
September	24	630**	4000**cc
		C_2 Horizon	
February[a]	16	9.5	-
June	25	61	-
July[b]	17	210**	40**cc
August	8.1	n.d.	250**
September	8.2	64**	1400**cc

a Within 24 hours after the winter oil spill.
b Within 24 hours after the summer oil spill.
** Significantly different from the control at 1% level.
cc Significantly different from the winter at 1% level.
n.d. Not determined.

TABLE C-5. 20°C ANAEROBIC BACTERIAL COUNTS ($\times 10^5$/ g soil) IN OILED AND UNOILED (CONTROL)PLOTS IN 1976

Sampling time	Control plot	Winter plot	Summer plot
		A_1 Horizon	
July[a]	40	72*	19**cc
September	13	60**	1200**cc
		C_2 Horizon	
July[a]	7.7	14**	9.9cc
September	4.8	11**	21**cc

a Within 24 hours after the summer oil spill.
* Significantly different from the control at 5% level.
** Significantly different from the control at 1% level.
cc Significantly different from the winter at 1% level.

TABLE C-6. 20°C IN VITRO SOIL RESPIRATION RATES (mg CO_2/24 HR/100 g soil) IN OILED AND UNOILED (CONTROL) PLOTS

Sampling time	Control plot	Winter plot	Summer plot
1976		A_1 Horizon	
February[a]	104.0	49.4**	-
July[b]	39.8	97.4**	146.0**cc
August	25.8	42.0**	88.2**cc
September	21.6	48.8**	121.0**cc
		C_2 Horizon	
February[a]	10.0	3.2**	-
July[b]	12.2	20.2**	15.0c
August	7.8	15.4**	9.0c
September	5.6	18.0**	33.6**cc
1977		A_1 Horizon	
June	61.6	90.8**	223.8**cc
July	40.5	55.7**	136.2**cc
August	65.2	54.0**	128.7**cc
		C_2 Horizon	
June	7.3	11.4**	37.5**cc
July	9.9	14.3**	17.9**cc
August	9.2	15.0**	19.8**cc
1978		A_1 Horizon	
June	41.0	68.8**	82.5**cc
July	11.1	17.2**	14.4*

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a Within 24 hours after the winter oil spill.

b Within 24 hours after the summer oil spill.

* Significantly different from the control at 5% level.

** Significantly different from the control at 1% level.

c Significantly different from the winter at 5% level.

cc Significantly different from the winter at 1% level.

TABLE C-7. pH OF SOIL COLLECTED FROM OILED AND UNOILED (CONTROL) PLOTS

Sampling time	Control plot	Winter plot	Summer plot
		A_1 Horizon	
1976			
February[a]	4.8	5.5	--
June	5.6	5.7	--
July[b]	5.5	6.2	6.1
August	5.4	5.7	5.5
September	5.2	5.7	5.7
		C_2 Horizon	
February[a]	5.2	5.2	--
June	5.0	5.5	--
July[b]	5.1	5.2	5.3
August	5.3	5.5	5.7
September	5.3	5.6	5.9
		A_1 Horizon	
1977			
June	5.2	4.9	5.3
July	4.8	5.1	5.4
August	5.1	5.5	5.6
		C_2 Horizon	
June	5.2	5.2	5.4
July	5.0	5.2	5.2
August	5.2	5.2	5.2
		A_1 Horizon	
1978			
June	4.9	5.4	5.2
July	4.8	5.2	5.3
		C_2 Horizon	
June	5.2	5.4	5.4
July	5.0	5.0	5.2

[a] Within 24 hours after the winter oil spill.
[b] Within 24 hours after the summer oil spill.

TABLE C-8. CORRELATION COEFFICIENTS AND REGRESSION EQUATIONS RELATING _IN VITRO_ SOIL RESPIRATION RATES TO BACTERIAL NUMBERS IN OILED AND UNOILED (CONTROL) PLOTS IN 1977[a].

Rregression equation [b]	Treatment	n	r
	4°C		
$Y = 6.67 + 2.06X$	Control	6	0.831*
$Y = 8.56 + 0.04X$	Winter	6	0.959**
$Y = 7.90 + 0.05X$	Summer	6	0.927**
$Y = 9.31 + 0.05X$	All	18	0.927**
	20°C		
$Y = 0.25 + 2.36X$	Control	6	0.959**
$Y = 12.71 + 0.12X$	Winter	6	0.938**
$Y = 2.28 + 0.12X$	Summer	6	0.990**
$Y = 19.34 + 0.11X$	All	18	0.943**

a June, July, August data in both soil horizons were used.
b Y = mg CO_2/24 hr/100g soil; $X = 10^6$ bacterial cells/g soil.

* Significant at 5% level.
** Significant at 1% level.

TABLE C-9. CORRELATION COEFFICIENTS AND REGRESSION EQUATIONS RELATING IN VITRO SOIL RESPIRATION RATES TO BACTERIAL NUMBERS IN OILED AND UNOILED (CONTROL) PLOTS IN 1976[a].

Regression equation [b]	Treatment	n	r
	4°C		
Y = 2.27 + 1.72X	Control	6	0.892*
Y = 7.10 + 0.03X	Winter	6	0.863*
Y = 8.60 + 0.05X	Summer	6	0.860*
Y = 6.09 + 0.05X	All	18	0.858**
	20°C		
Y = 0.05 + 1.45X	Control	6	0.980**
Y = 10.20 + 0.17X	Winter	6	0.952**
Y = 49.19 + 0.05X	Summer	6	0.466
Y = 30.51 + 0.08X	All	18	0.566*

a July, August and September data in both soil horizons were used.
b Y = mg CO_2/24 hr/100g soil; X = bacterial cells x 10^6/g soil.

* Significant at 5% level.
** Significant at 1% level.

TABLE C-10. CORRELATION COEFFICIENTS AND REGRESSION EQUATIONS RELATING *IN VITRO* SOIL RESPIRATION RATES TO FILAMENTOUS FUNGI PROPAGULE COUNTS IN OILED AND UNOILED (CONTROL) PLOTS IN 1976[a].

Regression equation [b]	Treatment	n	r
	4°C		
$Y = 3.47 + 0.04X$	Control	6	0.724
$Y = 5.47 + 0.53X$	Winter	6	0.822*
$Y = 7.52 + 0.30X$	Summer	6	0.806
$Y = 7.37 +).15X$	All	18	0.504*
	20°C		
$Y = 6.13 + 0.21X$	Control	6	0.980**
$Y = 34.91 + 0.25X$	Winter	5	0.277
$Y = 47.86 + 0.17X$	Summer	6	0.500
$Y = 28.39 + 0.21X$	All	17	0.520*

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[a] July, August, and September data in both soil horizons were used.

[b] Y = mg CO_2/24 hr/100 g soil; X = 10^4 filamentous fungal propagules/g soil.

* Significant at 5% level.

** Significant at 1% level.

TABLE C-11. CORRELATION COEFFICIENTS AND REGRESSION EQUATIONS RELATING IN VITRO SOIL RESPIRATION RATES TO FILAMENTOUS FUNGI PROPAGULE COUNTS IN OILED AND UNOILED (CONTROL) PLOTS IN 1977[a].

Regression equation [b]	Treatment	n	r
	4°C		
$Y = 2.82 + 0.17X$	Control	6	0.980**
$Y = 10.01 + 0.78X$	Winter	6	0.933**
$Y = 1.14 + 1.58X$	Summer	6	0.906*
$Y = 15.50 + 0.18X$	All	18	0.283
	20°C		
$Y = 6.72 + 0.23X$	Control	6	0.894*
$Y = -4.44 + 1.77X$	Winter	6	0.843*
$Y = 7.80 + 0.68X$	Summer	6	0.995**
$Y = 15.20 + 0.46X$	All	18	0.794**

[a] June, July and August data in both soil horizons were used.
[b] Y = mg CO_2/24 hr/100 g soil; $X = 10^4$ filamentous fungal propagules/g soil.

* Significant at 5% level.
** Significant at 1% level.

TABLE C-12. CORRELATION COEFFICIENTS AND REGRESSION EQUATIONS RELATING *IN VITRO* SOIL RESPIRATION RATES TO YEAST NUMBERS IN OILED AND UNOILED (CONTROL) PLOTS IN 1976[a].

Regression equation [b]	Treatment	n	r
	4°C		
$Y = 1.91 + 4.26X$	Control	6	0.710
$Y = 6.89 + 0.25X$	Winter	6	0.792
$Y = 7.45 + 0.04X$	Summer	6	0.853*
$Y = 7.62 + 0.04X$	All	18	0.837**
	20°C		
$Y = 14.26 + 1.12X$	Control	6	0.374
$Y = 29.47 + 0.09X$	Winter	6	0.382
$Y = 48.67 + 0.03X$	Summer	6	0.412
$Y = 31.12 + 0.04X$	All	18	0.545*

a July, August and September data in both soil horizons were used.

b Y = mg CO_2/24 hr/100 g soil; $X = 10^5$ yeast cells/g soil.

* Significant at 5% level.

** Significant at 1% level.

TABLE C-13. CORRELATION COEFFICIENTS AND REGRESSION EQUATIONS RELATING IN VITRO SOIL RESPIRATION RATES TO YEAST NUMBERS IN OILED AND UNOILED (CONTROL) PLOTS IN 1977[a].

Regression equation [b]	Treatment	n	r
	4°C		
Y = 5.49 + 3.46X	Control	6	0.781
Y = 9.03 + 0.72X	Winter	6	0.964**
Y = 8.19 +).51X	Summer	6	0.985**
Y = 9.96 + 0.50X	All	18	0.964**
	20°C		
Y = 6.04 + 11.01X	Control	6	0.800
Y = 8.52 + 0.20X	Winter	6	0.768
Y = 21.44 + 0.03X	Summer	6	0.768
Y = 31.43 + 0.03X	All	18	0.775**

a June, July and August data in both soil horizons were used.
b Y = mg CO_2/24 hr/100 g soil; X = 10^5 yeast cells/g soil.

** Significant at 1% level.

TABLE C-14. CORRELATION COEFFICIENTS AND REGRESSION EQUATIONS RELATING IN VITRO SOIL RESPIRATION RATES TO OIL CONCENTRATIONS IN OILED PLOTS IN 1976[a].

Regression equation [b]	Treatment	n	r
	4°C		
$Y = 5.58 + 1.53X$	Winter	6	0.879*
$Y = 9.85 + 1.84X$	Summer	6	0.607
$Y = 7.13 + 1.84X$	All	12	0.643*
	20°C		
$Y = 2.71 + 10.21X$	Winter	6	0.990**
$Y = 14.36 + 11.92X$	Summer	6	0.958**
$Y = 6.19 + 11.72X$	Both	12	0.945**

[a] July, August and September data in both soil horizons were used.
[b] Y = mg CO_2/24 hr/100 g soil; X = % oil in soil.

* Significant at 5% level.
** Significant at 1% level.

TABLE C-15. CORRELATION COEFFICIENTS AND REGRESSION EQUATIONS RELATING IN VITRO SOIL RESPIRATION RATES TO OIL CONCENTRATIONS IN OILED PLOTS IN 1976[a].

Regression equation [b]	Treatment	n	r
	4°C		
$Y = 5.62 + 2.17X$	Winter	6	0.980**
$Y = 11.29 + 2.85X$	Summer	6	0.970**
$Y = 6.60 + 2.89X$	Both	12	0.949**
	20°C		
$Y = 5.52 + 7.33X$	Winter	6	0.917**
$Y = 29.39 + 8.53X$	Summer	6	0.943**
$Y = 12.80 + 8.82X$	Both	12	0.922**

a June, July and August data in both soil horizons were used.
b Y = mg CO_2/24 hr/100 g soil; X = % oil in soil.

** Significant at 1% level.

TABLE C-16. CORRELATION COEFFICIENTS AND REGRESSION EQUATIONS RELATING BACTERIAL NUMBERS TO OIL CONCENTRATIONS IN OILED PLOTS IN 1976[a].

Regression equation [b]	Treatment	n	r
	4°C		
$Y = -51.29 + 54.11X$	Winter	6	0.997**
$Y = 161.37 + 9.06X$	Summer	6	0.164
$Y = 80.34 + 23.04X$	Both	12	0.421
	20°C		
$Y = -30.90 + 56.08X$	Winter	6	0.980**
$Y = 259.92 + 22.46X$	Summer	6	0.210
$Y = 124.15 + 35.14X$	Both	12	0.359

[a] July, August and September data in both soil horizons were used.
[b] $Y = 10^6$ bacterial cells/g soil; X = % oil in soil.

** Significant at 1% level.

TABLE C-17. CORRELATION COEFFICIENTS AND REGRESSION EQUATIONS RELATING BACTERIAL NUMBERS TO OIL CONCENTRATIONS IN OILED PLOTS IN 1977[a].

Regression equation [b]	Treatment	n	r
	4°C		
$Y = -56.40 + 47.76X$	Winter	6	0.975**
$Y = 108.04 + 55.20X$	Summer	6	0.964**
$Y = -4.70 + 57.30X$	Both	12	0.938**
	20°C		
$Y = -62.69 + 63.35X$	Winter	6	0.980**
$Y = 222.43 + 69.21X$	Summer	6	0.949**
$Y = 40.30 + 73.38X$	Both	12	0.917**

[a] June, July and August data in both soil horizons were used.
[b] $Y = 10^6$ bacterial cells/g soil; X = % oil in soil.

** Significant at 1% level.

TABLE C-18. CORRELATION COEFFICIENTS AND REGRESSION EQUATIONS RELATING BACTERIAL NUMBERS TO OIL CONCENTRATIONS IN OILED PLOTS IN 1978[a].

Regression equation [b]	Treatment	n	r
	4°C		
$Y = 12.77 + 21.05X$	Winter	4	0.995**
$Y = -7.64 + 36.56X$	Summer	4	0.889
$Y = -4.48 + 29.79X$	Both	8	0.854**
	20°C		
$Y = -8.98 + 41.92X$	Winter	4	1.000**
$Y = 4.35 + 58.40X$	Summer	4	0.975*
$Y = -8.47 + 50.96X$	Both	8	0.943**

[a] June and July data in both soil horizons were used.
[b] $Y = 10^6$ bacterial cells/g soil; X = % oil in soil.

* Significant at 5% level.
** Significant at 1% level.

TABLE C-19. CORRELATION COEFFICIENTS AND REGRESSION EQUATIONS RELATING BACTERIAL NUMBERS TO OIL CONCENTRATIONS IN OILED PLOTS IN 1978[a].

Regression equation [b]	Treatment	n	r
	4°C		
$Y = -4.20 + 2.49X$	Winter	6	0.938**
$Y = 8.37 + 1.55X$	Summer	6	0.922**
$Y = 2.61 + 1.82X$	Both	12	0.889**
	20°C		
$Y = 8.71 + 3.30X$	Winter	6	0.812*
$Y = 35.65 + 11.95X$	Summer	6	0.911*
$Y = 3.08 + 11.73X$	Both	12	0.831**

[a] June, July and August data in both soil horizons were used.
[b] $Y = 10^4$ filamentous fungal propagules/g soil; X = % oil in soil.

* Significant at 5% level.
** Significant at 1% level.

TABLE C-20. CORRELATION COEFFICIENTS AND REGRESSION EQUATIONS RELATION FILAMENTOUS FUNGAL PROPAGULE COUNTS TO OIL CONCENTRATIONS IN OILED PLOTS IN 1978[a].

Regression equation[b]	Treatment	n	r
	4°C		
Y = 3.29 + 1.66X	Winter	4	0.990**
Y = 7.05 + 4.55X	Summer	4	0.990**
Y = 4.14 + 3.23X	Both	8	0.762*
	20°C		
Y = -68.76 + 57.57X	Winter	4	0.985*
Y = 33.54 + 43.63X	Summer	3	0.990
Y = -22.62 + 50.70X	Both	7	0.975**

[a] June and July data in both soil horizons were used.
[b] $Y = 10^4$ filamentous fungal propagules/g soil; X = % oil in soil.

* Significant at 5% level.
** Significant at 1% level.

TABLE C-21. CORRELATION COEFFICIENTS AND REGRESSION EQUATIONS RELATION YEAST NUMBERS TO OIL CONCENTRATIONS IN OILED PLOTS IN 1977[a].

Regression equation [b]	Treatment	n	r
	4°C		
$Y = -4.22 + 2.92X$	Winter	6	0.980**
$Y = 5.25 + 5.56X$	Summer	6	0.995**
$Y = -5.44 + 5.51X$	Both	12	0.938**
	20°C		
$Y = 88.42 + 14.56X$	Winter	6	0.480
$Y = 1274.73 + 118.41X$	Summer	6	0.574
$Y = 366.12 + 129.73X$	Both	12	0.539

a June, July and August data in both soil horizons were used.
b $Y = 10^5$ yeast cells/g soil; X = % oil in soil.

** Significant at 1% level.

TABLE C-22. CORRELATION COEFFICIENTS AND REGRESSION EQUATIONS RELATING YEAST NUMBERS TO OIL CONCENTRATIONS IN OILED PLOTS IN 1978[a].

Regression equation [b]	Treatment	n	r
	4°C		
$Y = 5.62 + 2.73X$	Winter	4	0.894
$Y = -3.30 + 22.72X$	Summer	4	0.889
$Y = -7.27 + 13.87X$	Both	8	0.616
	20°C		
$Y = -312.54 + 377.99X$	Winter	4	0.998**
$Y = 78.97 + 304.45X$	Summer	4	0.998**
$Y = -72.12 + 334.38X$	Both	8	0.992**

[a] June and July data in both soil horizons were used.
[b] $Y = 10^5$ yeast cells/g soil; X = % oil in soil.

** Significant at 1% level.